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=> d his

     (FILE 'HOME' ENTERED AT 12:03:10 ON 24 APR 2003)

     FILE 'HCAPLUS' ENTERED AT 12:03:33 ON 24 APR 2003
L1         12901 S AFFINITY (L) (CHROMAT? OR SEPN OR SEPARATION?)
L2       1173360 S PROTEIN#
L3         14558 S ASN OR ASPARAGINE

     FILE 'REGISTRY' ENTERED AT 12:04:07 ON 24 APR 2003
               E ASPARAGINE/CN
L4             2 S E3

     FILE 'HCAPLUS' ENTERED AT 12:04:17 ON 24 APR 2003
L5         12476 S L4
L6         12476 S L4 OR L5
L7          3238 S L6 AND L2
L8            10 S L1 AND L7
L9           242 S (MODIF? OR ALTER?) (L) L3
L10            6 S L9 AND L1
L11       127082 S LIGAND#
L12            9 S L9 AND L11
L13           23 S L12 OR L10 OR L8
L14         2489 S (ALKALINE OR ALK#) (L) RESIST?
L15            1 S L14 AND L9
L16            3 S L6 AND L11 AND L1
L17            1 S L6 AND L14
L18           24 S L13 OR L15 OR L16 OR L17
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=> fil hcaplus
FILE 'HCAPLUS' ENTERED AT 12:10:02 ON 24 APR 2003
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FILE COVERS 1907 - 24 Apr 2003  VOL 138 ISS 17
FILE LAST UPDATED: 23 Apr 2003  (20030423/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

'OBI' IS DEFAULT SEARCH FIELD FOR 'HCAPLUS' FILE

=> d his

     (FILE 'HOME' ENTERED AT 12:03:10 ON 24 APR 2003)

     FILE 'HCAPLUS' ENTERED AT 12:03:33 ON 24 APR 2003
L1         12901 S AFFINITY (L) (CHROMAT? OR SEPN OR SEPARATION?)
L2       1173360 S PROTEIN#
L3         14558 S ASN OR ASPARAGINE

     FILE 'REGISTRY' ENTERED AT 12:04:07 ON 24 APR 2003
                E ASPARAGINE/CN
L4             2 S E3

     FILE 'HCAPLUS' ENTERED AT 12:04:17 ON 24 APR 2003
L5         12476 S L4
L6         12476 S L4 OR L5
L7          3238 S L6 AND L2
L8            10 S L1 AND L7
L9           242 S (MODIF? OR ALTER?) (L) L3
L10            6 S L9 AND L1
L11       127082 S LIGAND#
L12            9 S L9 AND L11
L13           23 S L12 OR L10 OR L8
L14         2489 S (ALKALINE OR ALK#) (L) RESIST?
L15            1 S L14 AND L9
L16            3 S L6 AND L11 AND L1
L17            1 S L6 AND L14
L18           24 S L13 OR L15 OR L16 OR L17

     FILE 'HCAPLUS' ENTERED AT 12:10:02 ON 24 APR 2003

=> d que l24
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L24 NOT FOUND

=> s que l18
MISSING OPERATOR

=> d que l18
L1 12901 SEA FILE=HCAPLUS ABB=ON PLU=ON AFFINITY/OBI (L) (CHROMAT?/OBI OR SEPN/OBI OR SEPARATION?/OBI)
L2 1173360 SEA FILE=HCAPLUS ABB=ON PLU=ON PROTEIN#/OBI
L3 14558 SEA FILE=HCAPLUS ABB=ON PLU=ON ASN/OBI OR ASPARAGINE/OBI
L4 2 SEA FILE=REGISTRY ABB=ON PLU=ON ASPARAGINE/CN
L5 12476 SEA FILE=HCAPLUS ABB=ON PLU=ON L4
L6 12476 SEA FILE=HCAPLUS ABB=ON PLU=ON L4 OR L5
L7 3238 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND L2
L8 10 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND L7
L9 242 SEA FILE=HCAPLUS ABB=ON PLU=ON (MODIF?/OBI OR ALTER?/OBI) (L) L3
L10 6 SEA FILE=HCAPLUS ABB=ON PLU=ON L9 AND L1
L11 127082 SEA FILE=HCAPLUS ABB=ON PLU=ON LIGAND#/OBI
L12 9 SEA FILE=HCAPLUS ABB=ON PLU=ON L9 AND L11
L13 23 SEA FILE=HCAPLUS ABB=ON PLU=ON L12 OR L10 OR L8
L14 2489 SEA FILE=HCAPLUS ABB=ON PLU=ON (ALKALINE/OBI OR ALK#/OBI) (L) RESIST?/OBI
L15 1 SEA FILE=HCAPLUS ABB=ON PLU=ON L14 AND L9
L16 3 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND L11 AND L1
L17 1 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND L14
L18 24 SEA FILE=HCAPLUS ABB=ON PLU=ON L13 OR L15 OR L16 OR L17

=> d .ca l18 1-24

L18 ANSWER 1 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:254306 HCAPLUS
DOCUMENT NUMBER: 138:250704
TITLE: Water-soluble Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatography** and use in glucose assay and sensor
INVENTOR(S): Hayade, Hiroshi
PATENT ASSIGNEE(S): Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003093071	A2	20030402	JP 2001-294846	20010926
WO 2003027294	A1	20030403	WO 2002-JP9943	20020926

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA,

UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 2001-294846 A 20010926

AB A water-sol. Acinetobacter calcoaceticus pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) mutant for easier purifn. with anion exchange chromatog., is disclosed. Recombinant expression of those PQQGDH mutants, glucose assay kit and glucose sensor contg. them are claimed. Based on the three-dimensional structure of water-sol. PQQGDH, a series of mutants having a glutamine, asparagine and threonine on the surface substituted with the arginine, was constructed. Q209R/N240R/T389R triple mutant was engineered, expressed in E. coli, and purified on anion exchange chromatog. column. Km and Vmax values for glucose and other substrates of the mutant were comparable to those for the wild type. A glucose assay kit and glucose sensor were developed to measure glucose between 1 to 12 mM.

IC ICM C12N015-09
ICS C12M001-34; C12N001-15; C12N001-19; C12N001-21; C12N005-10; C12N009-04; C12R001-01

CC 7-1 (Enzymes)
Section cross-reference(s): 3, 9

IT Escherichia coli
(recombinant expression in; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

IT Mutagenesis
(site-directed, substitution; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

IT Acinetobacter calcoaceticus
Anion exchange liquid **chromatography**
Enzyme kinetics
Genetic engineering
Glucose sensors
Molecular cloning
Protein engineering
Test kits
(water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

IT 74-79-3, L-Arginine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(substitution of Glu, Asp, Thr with; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

IT 56-85-9, L-Glutamine, biological studies **70-47-3**, L-Asparagine, biological studies 72-19-5, L-Threonine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(substitution of, with arginine; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

IT 502955-91-1 502955-92-2 502955-93-3 502955-94-4
RL: PRP (Properties)
(unclaimed nucleotide sequence; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)
IT 502955-90-0
RL: PRP (Properties)
(unclaimed **protein** sequence; water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)
IT 50-99-7, D-Glucose, analysis
RL: ANT (Analyte); ANST (Analytical study)
(water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)
IT 81669-60-5P, Pyrroloquinoline quinone glucose dehydrogenase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); CAT (Catalyst use); PRP (Properties); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(water-sol. Acinetobacter pyrroloquinoline quinone glucose dehydrogenase mutant with improved **affinity** for anion exchange **chromatog.** and use in glucose assay and sensor)

L18 ANSWER 2 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:202771 HCAPLUS
DOCUMENT NUMBER: 138:233996
TITLE: Identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use
INVENTOR(S): Schneewind, Olaf; Mazmanian, Sarkis N.; Liu, Gwen; Ton-That, Hong
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 130 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003020885	A2	20030313	WO 2002-US26320	20020815
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003022178	A1	20030130	US 2001-933999	20010821

PRIORITY APPLN. INFO.: US 2001-312738P P 20010815

US 2001-933999 A 20010821
US 2002-219700 A 20020814
US 1999-292437 A2 19990415

AB The present invention is directed to sortase-transamidase enzyme from Gram-pos. bacteria, particularly the products of the surface protein sorting genes (srtA and srtB) of Staphylococcus aureus, and methods for their use. The nucleotide sequences of the genes srtA and srtB of S. aureus and the amino acid sequences of the encoded proteins are disclosed. The enzyme has a mol. wt. of 23.539 (SrtA) or 29.076 (SrtB) kDa includes: (1) a motif of LPX3X4G or NPQ/KTN/G; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two pos. charged residues carboxyl to the substantially hydrophobic domain, at least one of the two pos. charged residues being arginine, the two pos. charged residues being located at residues 31-33 from the motif, wherein X3 is any of the twenty naturally-occurring L-amino acids, and X4 us alanine, serine, or threonine. Variants of the enzyme, methods for cloning the gene encoding the enzyme and expressing the cloned gene, and methods of use of the enzyme, including for screening for antibiotics and for display of proteins or peptides on the surfaces of Gram-pos. bacteria, are also disclosed.

IC ICM C12N

CC 7-5 (Enzymes)
Section cross-reference(s): 1, 3, 10, 15

ST Staphylococcus gene srtA srtB sortase transamidase sequence drug screening; Gram pos bacteria sortase **protein** display therapeutics diagnosis

IT **Proteins**
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(MBP (maltose-binding **protein**), chimeric **protein** contg.; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Cell wall
(chimeric **protein** conjugated to; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Antigens
Haptens
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(conjugates, with sortase-contg. chimeric **protein**; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Antibacterial agents
DNA sequences
Drug screening
Gram-positive bacteria (Firmicutes)
Molecular cloning
Protein motifs
Protein sequences
Staphylococcus aureus
(identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT **Affinity chromatographic** stationary phases

(in drug screening assay; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Crosslinking
(of antigen or hapten with sortase-contg. chimeric **protein**; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Genetic methods
(**protein** display; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT Fusion **proteins** (chimeric **proteins**)
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(sortase-contg., in **protein** display method; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT 9013-20-1, Streptavidin 50812-37-8, Glutathione S-transferase
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(chimeric **protein** contg.; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT 56-45-1, L-Serine, biological studies 56-84-8, L-Aspartic acid, biological studies 56-85-9, L-Glutamine, biological studies 56-86-0, L-Glutamic acid, biological studies 61-90-5, L-Leucine, biological studies **70-47-3**, L-Asparagine, biological studies 72-18-4, L-Valine, biological studies 72-19-5, L-Threonine, biological studies 73-32-5, L-Isoleucine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(conservative substitution mutation; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT 74-79-3, Arginine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**protein** motif contg.; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

IT 501737-74-2 501737-75-3 501737-76-4 501737-77-5 501737-78-6
501737-79-7 501737-80-0 501737-81-1 501737-82-2 501737-83-3
501737-84-4 501737-85-5 501737-86-6 501737-87-7 501737-88-8
501737-89-9 501737-90-2 501737-91-3 501737-92-4 501737-93-5
501737-94-6 501737-95-7 501737-98-0 501737-99-1 501738-00-7
501738-02-9 501738-03-0
RL: PRP (Properties)
(unclaimed **protein** sequence; identification, cloning, and sequencing of Staphylococcus aureus SrtA and SrtB sortase isoenzymes for drug screening, therapeutic and diagnostic use)

L18 ANSWER 3 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:575099 HCAPLUS
DOCUMENT NUMBER: 137:137275
TITLE: Differential labeling for quantitative analysis of complex **protein** mixtures
INVENTOR(S): Haynes, Paul; Wei, Jing; Yates, John; Andon, Nancy
PATENT ASSIGNEE(S): Syngenta Participation Ag, USA
SOURCE: PCT Int. Appl., 79 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002059144	A2	20020801	WO 2002-US2487	20020125

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-264576P P 20010126
US 2001-305232P P 20010713

OTHER SOURCE(S): MARPAT 137:137275

AB The invention concerns a method of simultaneously identifying and detg. the levels of expression of cysteine-contg. proteins in normal and perturbed cells, a method for proteomic anal., a process for prepg. fusion proteins, and compds. and reagents related thereto. This invention provides methods and reagents that can be employed in proteome anal. which overcome the limitations inherent in traditional techniques The basic approach described can be employed for the quant. anal. of protein expression in complex samples (such as cells, tissues, and fractions thereof), the detection and quantitation of specific proteins in complex samples, and the quant. measurement of specific enzymic activities in complex samples. We have designed trifunctional synthetic peptide based reagents that can be used for reducing the complexity of peptide mixts. by labeling peptides with iodoacetamido groups and then selectively enriching only those peptides contg. labeled cysteine residues. Embodiments of this invention provide anal. reagents and mass spectrometry-based methods using these reagents for the rapid and quant. anal. of proteins or protein function in mixts. of proteins. The anal. method can be used for qual. and particularly for quant. anal. of global protein expression profiles in cells and tissues, i.e., the quant. anal. of proteomes.

IC ICM C07K007-04
ICS C07K001-00; G01N033-68; C12Q001-37
CC 9-16 (Biochemical Methods)
Section cross-reference(s): 6
ST **protein** fusion motif cleavage cysteine label mass spectrometry
IT **Protein** motifs
(amino acid reactive; differential labeling for quant. anal. of complex **protein** mixts.)
IT Liquid chromatography
(charge differentiation; differential labeling for quant. anal. of complex **protein** mixts.)
IT Chelating agents
(column; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Proteins**
RL: ANT (Analyte); PRP (Properties); ANST (Analytical study)
(cysteine-contg.; differential labeling for quant. anal. of complex **protein** mixts.)

IT Acyl groups
Affinity chromatography
Chemicals
Databases
Epitopes
Heat
Hydrophilicity
Hydrophobicity
Immobilization, molecular
Labels
Light
Magnetic materials
Mass spectrometry
Molecular association
Molecular weight
Polarity
Protein degradation
Protein sequences
Separation
Size-exclusion chromatography
Staphylococcus aureus
Washing
Wavelength
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Proteome
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Acids, uses
RL: NUU (Other use, unclassified); USES (Uses)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Bases, uses
RL: NUU (Other use, unclassified); USES (Uses)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Reagents
RL: NUU (Other use, unclassified); USES (Uses)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Oligonucleotides
RL: NUU (Other use, unclassified); PRP (Properties); USES (Uses)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Amino acids, properties
RL: PRP (Properties)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Fusion **proteins** (chimeric **proteins**)
RL: SPN (Synthetic preparation); PREP (Preparation)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT Fracture (materials)
(heterolytically; differential labeling for quant. anal. of complex **protein** mixts.)

IT Fracture (materials)
(homolytically; differential labeling for quant. anal. of complex

protein mixts.)
IT **Protein** motifs
(magnetic; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(mass variance; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(metal chelator; differential labeling for quant. anal. of complex **protein** mixts.)
IT Liquid chromatography
Mass spectrometry
(multidimensional; differential labeling for quant. anal. of complex **protein** mixts.)
IT Liquid chromatography
(one dimensional; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(photocleavage linker; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(protease cleavage; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(restriction enzyme cleavage; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(specific oligonucleotide sequence; differential labeling for quant. anal. of complex **protein** mixts.)
IT **Protein** motifs
(thermal cleavage; differential labeling for quant. anal. of complex **protein** mixts.)
IT Liquid chromatography
(three dimensional; differential labeling for quant. anal. of complex **protein** mixts.)
IT Mass spectrometry
(two-dimensional; differential labeling for quant. anal. of complex **protein** mixts.)
IT 158760-86-2, ENLYFQG 444196-30-9D, acyl derivs. 444196-31-0D, acyl derivs. 444196-32-1D, acyl derivs. 444196-33-2D, acyl derivs. 444196-34-3D, acyl derivs. 444196-35-4D, acyl derivs. 444196-36-5D, acyl derivs. 444196-37-6D, acyl derivs. 444196-38-7D, acyl derivs. 444196-39-8D, acyl derivs. 444196-40-1D, acyl derivs. 444196-41-2D, acyl derivs. 444196-42-3D, acyl derivs. 444196-43-4D, acyl derivs. 444196-44-5D, acyl derivs.
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; differential labeling for quant. anal. of complex **protein** mixts.)
IT 52-90-4, L-Cysteine, biological studies 56-45-1, L-Serine, biological studies 56-84-8, L-Aspartic acid, biological studies 56-85-9, L-Glutamine, biological studies 56-86-0, L-Glutamic acid, biological studies 56-87-1, L-Lysine, biological studies 60-18-4, L-Tyrosine, biological studies 63-68-3, L-Methionine, biological studies **70-47-3**, L-Asparagine, biological studies 71-00-1, L-Histidine, biological studies 72-19-5, L-Threonine, biological studies 73-22-3, L-Tryptophan, biological studies 74-79-3, L-Arginine, biological studies 144-48-9D, Iodoacetamide, coupled with lysine, ornithine or arginine

147-85-3, L-Proline, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT 444196-45-6DP, acyl derivs. 444196-46-7DP, acyl derivs. 444196-47-8DP, acyl derivs. 444196-48-9DP, acyl derivs. 444196-49-0DP, acyl derivs. 444196-50-3DP, acyl derivs. 444196-51-4DP, acyl derivs. 444196-52-5DP, acyl derivs.
RL: BSU (Biological study, unclassified); PRP (Properties); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT 7440-02-0, Nickel, uses 9001-75-6, Pepsin 9001-92-7, Protease 9002-07-7, Trypsin 9004-07-3, Chymotrypsin 9073-78-3, Thermolysin 9075-08-5, Restriction enzyme 55576-49-3, Endoproteinase Asp-N 115775-22-9, TEV protease 123175-81-5, Endoproteinase Arg-C
RL: NUU (Other use, unclassified); USES (Uses)
(differential labeling for quant. anal. of complex **protein** mixts.)

IT 444945-74-8 444945-75-9 444945-77-1 444945-79-3 444945-80-6
RL: PRP (Properties)
(unclaimed **protein** sequence; differential labeling for quant. anal. of complex **protein** mixts.)

IT 92000-76-5 321888-23-7 321888-25-9 321888-26-0 321888-28-2
321888-31-7 350690-98-1 437984-21-9 437984-26-4 437984-31-1
444875-72-3 444875-73-4 444875-74-5 444875-75-6 444875-76-7
444875-77-8 444875-78-9 444875-79-0 444875-80-3 444875-81-4
444875-82-5 444875-83-6 444875-84-7 444875-85-8 444875-86-9
444875-87-0 444875-88-1 444875-89-2 444875-90-5 444875-91-6
444875-92-7 444875-93-8 444875-94-9 444875-95-0 444875-96-1
444875-97-2 444875-98-3 444875-99-4 444876-00-0 444876-01-1
444876-02-2 444876-03-3 444876-04-4 444876-05-5 444876-06-6
444876-07-7 444876-08-8 444876-09-9 444876-10-2 444876-11-3
444876-12-4 444876-13-5 444876-14-6 444876-15-7 444876-16-8
444876-17-9 444876-18-0 444876-19-1 444876-20-4 444876-21-5
444876-22-6 444876-23-7 444876-24-8 444876-25-9 444876-26-0
444876-27-1 444876-28-2 444876-29-3 444876-30-6 444876-31-7
444876-32-8 444876-33-9 444876-34-0 444876-35-1 444876-36-2
444876-37-3 444876-38-4 444876-39-5 444876-40-8 444876-41-9
444876-42-0 444876-43-1 444876-44-2 444876-45-3 444876-46-4
444876-47-5 444876-48-6 444876-49-7 444876-50-0 444876-51-1
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444876-57-7 444876-58-8 444876-59-9 444876-60-2 444876-61-3
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444876-67-9 444876-68-0 444876-69-1 444876-70-4 444876-71-5
444876-72-6 444876-73-7 444876-74-8 444876-75-9 444876-76-0
444876-77-1 444876-78-2 444876-79-3 444876-80-6 444876-81-7
444876-82-8 444876-83-9 444876-84-0 444876-85-1 444876-86-2
444876-87-3 444876-88-4 444876-89-5 444876-90-8 444876-91-9
444876-92-0 444876-93-1 444876-94-2 444876-95-3 444876-96-4
444876-97-5 444876-98-6 444876-99-7 444877-00-3 444877-01-4
444877-02-5 444877-03-6 444877-04-7 444877-05-8 444877-06-9
444877-07-0 444877-08-1 444877-09-2 444877-10-5 444877-11-6
444877-12-7 444877-13-8 444877-14-9 444877-15-0 444877-16-1
444877-17-2 444877-18-3 444877-19-4 444877-20-7 444877-21-8
444877-22-9 444877-23-0 444877-24-1 444877-25-2 444877-26-3
444877-27-4 444877-28-5 444877-29-6 444877-30-9 444877-31-0

444877-32-1 444877-33-2 444877-34-3 444877-35-4 444877-36-5
444877-37-6 444877-38-7 444877-39-8 444877-40-1 444877-41-2
444877-42-3 444877-43-4 444877-44-5 444877-45-6 444877-46-7
444877-47-8 444877-48-9 444877-49-0 444877-50-3 444877-51-4
444877-52-5 444877-53-6 444877-54-7 444877-55-8 444877-56-9
444877-57-0 444877-58-1 444877-59-2 444877-60-5 444877-61-6
444877-62-7 444877-63-8 444877-64-9 444877-65-0 444877-66-1
444877-67-2 444877-68-3 444877-69-4 444877-70-7 444877-71-8
444877-72-9 444877-73-0 444877-74-1 444877-75-2 444877-76-3
444877-77-4 444877-78-5 444877-79-6 444877-80-9 444877-81-0
444877-82-1 444877-83-2 444877-84-3 444877-85-4 444877-86-5
444877-87-6 444877-88-7 444877-89-8 444877-90-1 444877-91-2
444877-92-3 444877-93-4 444877-94-5 444877-95-6 444877-96-7
444877-97-8 444877-98-9 444877-99-0 444878-00-6 444878-01-7
RL: PRP (Properties)
(unclaimed sequence; differential labeling for quant. anal. of complex **protein** mixts.)

IT 444878-02-8 444878-03-9 444878-04-0 444878-05-1 444878-06-2
444878-07-3 444878-08-4 444878-09-5 444878-10-8 444878-11-9
444878-12-0 444878-13-1 444878-14-2 444878-15-3 444878-16-4
444878-17-5 444878-18-6 444878-19-7 444878-20-0 444878-21-1
444878-22-2 444878-23-3 444878-24-4 444878-25-5 444945-78-2
RL: PRP (Properties)
(unclaimed sequence; differential labeling for quant. anal. of complex **protein** mixts.)

L18 ANSWER 4 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:875227 HCAPLUS
DOCUMENT NUMBER: 136:34272
TITLE: Engineering affinity **ligands** for macromolecules
INVENTOR(S): Maclennan, John Moore; Ladner, Robert Charles
PATENT ASSIGNEE(S): Dyax Corp., USA
SOURCE: U.S., 48 pp., Cont.-in-part of U.S. Ser. No. 619,885.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6326155	B1	20011204	US 1997-821498	19970321
CA 2248243	AA	19970925	CA 1997-2248243	19970320
CN 1219240	A	19990609	CN 1997-194698	19970320
US 2002076728	A1	20020620	US 2001-957607	20010919

PRIORITY APPLN. INFO.: US 1996-619885 A2 19960320
US 1997-821498 A3 19970321

AB The invention concerns A method for obtaining highly specific and tailored ligands suitable for purifying a particular product target or for eliminating particular target impurities in a feed stream. Engineered affinity ligands according to the invention will bind a target with high specificity at a preselected binding condition and release the target at a preselected elution condition. The ligands are isolated by contacting a target with a multiplicity of polypeptides derived through variegation of the structure of a candidate binding domain, the variants (or analogs) including polypeptides favoring binding to the target under desired binding conditions and release from the target under elution conditions,

where the binding and elution conditions differ according to one or more parameters, such as pH, temp., concn. of salt or vol. % of an org. solvent.

IC G01N033-53; C12Q001-68; A61K038-16
NCL 435007100
CC 9-3 (Biochemical Methods)
Section cross-reference(s): 16, 63
ST **affinity chromatog ligand protein**
phage display library genetic method
IT Immunoglobulins
RL: BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(G; engineering affinity **ligands** for macromols.)
IT Gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(III; engineering affinity **ligands** for macromols.)
IT Denaturants
(chaotropic; engineering affinity **ligands** for macromols.)
IT **Affinity chromatography**
Anions
Bacteriophage
Cations
Chelating agents
Coliphage M13
Dielectric constant
Drugs
Genetic methods
Hydrogen bond
Immobilization, molecular
Ionic strength
Phage display library
Purification
Surface tension
Temperature
Temperature effects, biological
pH
(engineering **affinity ligands** for macromols.)
IT DNA
RL: ANT (Analyte); ANST (Analytical study)
(engineering affinity **ligands** for macromols.)
IT Salts, uses
RL: NUU (Other use, unclassified); USES (Uses)
(engineering affinity **ligands** for macromols.)
IT **Ligands**
RL: NUU (Other use, unclassified); SPN (Synthetic preparation); PREP (Preparation); USES (Uses)
(engineering affinity **ligands** for macromols.)
IT Biopolymers
Proteins
RL: PUR (Purification or recovery); PREP (Preparation)
(engineering affinity **ligands** for macromols.)
IT Gene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression; engineering affinity **ligands** for macromols.)
IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serum; engineering affinity **ligands** for macromols.)
IT 197528-41-9P

RL: CPN (Combinatorial preparation); SPN (Synthetic preparation); CMBI (Combinatorial study); PREP (Preparation)
(amino acid sequence; engineering affinity **ligands** for macromols.)

IT 197157-35-0P 197157-36-1P 197157-37-2P 197157-38-3P 197157-39-4P
197157-40-7P 197157-41-8P 197157-42-9P 197157-43-0P 197157-44-1P
197157-45-2P 197157-46-3P 197157-47-4P 197157-48-5P 197157-49-6P
197157-50-9P 197157-51-0P 197157-52-1P 197157-53-2P 197157-54-3P
197317-09-2P 197528-10-2P
RL: CPN (Combinatorial preparation); SPN (Synthetic preparation); CMBI (Combinatorial study); PREP (Preparation)
(engineering affinity **ligands** for macromols.)

IT 56-45-1, L-Serine, properties 56-84-8, L-Aspartic acid, properties
56-85-9, L-Glutamine, properties 56-86-0, L-Glutamic acid, properties
56-87-1, L-Lysine, properties 60-18-4, L-Tyrosine, properties
70-47-3, L-Asparagine, properties 71-00-1, L-Histidine, properties 72-19-5, L-Threonine, properties 74-79-3, L-Arginine, properties
RL: CPS (Chemical process); CST (Combinatorial study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); CMBI (Combinatorial study); PROC (Process)
(engineering affinity **ligands** for macromols.)

IT 7647-14-5, Sodium chloride (NaCl), uses
RL: CPS (Chemical process); NUU (Other use, unclassified); PEP (Physical, engineering or chemical process); PROC (Process); USES (Uses)
(engineering affinity **ligands** for macromols.)

IT 57-13-6, Urea, processes 60-00-4, EDTA, processes
RL: CPS (Chemical process); PEP (Physical, engineering or chemical process); PROC (Process)
(engineering affinity **ligands** for macromols.)

IT 71-50-1, Acetate, properties 113-21-3, Lactate, properties 126-44-3, Citrate, properties 7439-95-4, Magnesium, properties 7440-50-8, Copper, properties 7440-66-6, Zinc, properties 7440-70-2, Calcium, properties 14265-44-2, Phosphate, properties 14808-79-8, Sulfate, properties 16887-00-6, Chloride, properties
RL: CPS (Chemical process); PEP (Physical, engineering or chemical process); PRP (Properties); PROC (Process)
(engineering affinity **ligands** for macromols.)

IT 197528-40-8P
RL: CPN (Combinatorial preparation); SPN (Synthetic preparation); CMBI (Combinatorial study); PREP (Preparation)
(nucleic acid sequence; engineering affinity **ligands** for macromols.)

IT 280746-28-3
RL: PRP (Properties)
(unclaimed **protein** sequence; engineering affinity **ligands** for macromols.)

IT 197317-09-2 280746-28-3 379722-71-1 380151-87-1 380152-15-8
380152-35-2 380152-49-8
RL: PRP (Properties)
(unclaimed sequence; engineering affinity **ligands** for macromols.)

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 5 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:406859 HCAPLUS
DOCUMENT NUMBER: 135:190561

TITLE: Glycosylation of human CRLR at Asn123 is required for **ligand** binding and signaling
AUTHOR(S): Kamitani, S.; Sakata, T.
CORPORATE SOURCE: Shionogi Research Laboratories, Shionogi and Co. Ltd., Osaka, Settsu-shi, 566-0022, Japan
SOURCE: Biochimica et Biophysica Acta (2001), 1539(1-2), 131-139
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Calcitonin receptor-like receptor (CRLR) constitutes either a CGRP receptor when complexed with receptor activity-modifying protein 1 (RAMP1) or an adrenomedullin receptor when complexed with RAMP2 or RAMP3. RAMP proteins modify the glycosylation status of CRLR and det. their receptor specificity; when treated with tunicamycin, a glycosylation inhibitor, CHO-K1 cells constitutively expressing both RAMP2 and CRLR lost the capacity to bind adrenomedullin. Similarly, in HEK293 EBNA cells constitutively expressing RAMP1/CRLR receptor complex CGRP binding was remarkably inhibited. Which ever RAMP protein was co-expressing with CRLR, the ligand binding was sensitive to tunicamycin. There are three putative Asn-linked glycosylation sites in the extracellular, N-terminal domain of CRLR at positions 66, 118 and 123. Anal. of CRLR mutants in which Gln was substituted for selected Asn residues showed that glycosylation of Asn 123 is required for both the binding of adrenomedullin and the transduction of its signal. Substituting Asn 66 or Asn 118 had no effect. FACS anal. of cells expressing FLAG-tagged CRLRs showed that disrupting Asn-linked glycosylation severely affected the transport of the CRLR protein to the cell surface on N66/118/123Q mutant, and slightly reduced the level of the cell surface expression of N123Q mutant compared with wild-type CRLR. But other single mutants (N66Q, N118Q) had no effect for other single mutants. The authors' data shows that glycosylation of Asn 66 and Asn 118 is not essential for ligand binding, signal transduction and cell surface expression, and Asn 123 is important for ligand binding and signal transduction rather than cell surface expression. It thus appears that glycosylation of Asn 123 is required for CRLR to assume the appropriate conformation on the cell surface through its interaction with RAMPs.

CC 2-2 (Mammalian Hormones)

ST adrenomedullin receptor CRLR glycosylation asparagine structure activity signal transduction; structure activity relationship **ligand** binding adrenomedullin CGRP CRLR

IT G protein-coupled receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(CRLR (calcitonin receptor-like receptor); glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(RAMP1 (receptor-activity-**modifying** protein 1); glycosylation of human calcitonin receptor-like receptor at **asparagine** 123 is required for **ligand** binding and signaling)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(RAMP2 (receptor-activity-**modifying** protein 2); glycosylation

of human calcitonin receptor-like receptor at **asparagine** 123 is required for **ligand** binding and signaling)

IT Hormone receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(adrenomedullin; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Glycosylation
(biol.; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Signal transduction, biological
(glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Protein motifs
(glycosylation site; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Structure-activity relationship
(**ligand**-binding; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT Structure-activity relationship
(signal-transducing; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT 90954-53-3, human CGRP 161383-47-7, Rat adrenomedullin
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT 60-92-4, CAMP
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

IT 70-47-3, L-Asparagine, biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(residue 123; glycosylation of human calcitonin receptor-like receptor at asparagine 123 is required for **ligand** binding and signaling)

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 6 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:137384 HCAPLUS
DOCUMENT NUMBER: 134:188942
TITLE: Genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for bioseparations and methods for determining critical residues for varying cleavage activity
INVENTOR(S): Belfort, Marlene; Belfort, Georges; Derbyshire, Vicky; Wood, David; Wu, Wei
PATENT ASSIGNEE(S): Health Research Institute, USA
SOURCE: PCT Int. Appl., 103 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001012820	A1	20010222	WO 2000-US22581	20000817

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-149257P P 19990817

AB A self-cleaving element for use in biosepns. has been derived from a naturally occurring, 43 kDa protein splicing element (intein) through a combination of protein engineering and random mutagenesis. A mini-intein (18 kDa) previously engineered for reduced size had compromised activity and was therefore subjected to random mutagenesis and genetic selection. In one selection a mini-intein was isolated with restored splicing activity, while in another, a mutant was isolated with enhanced, pH-sensitive C-terminal cleavage activity. The enhanced cleavage mutant has utility in affinity fusion-based protein purifn. The enhanced splicing mutant has utility in purifn. of proteins such as toxic proteins, for example, by inactivation with the intein in a specific region and controllable splicing. These mutants also provide new insights into the structural and functional roles of some conserved residues in protein splicing. Thus, disclosed and claimed are: a genetic system and self-cleaving inteins therefrom; biosepns. employing same; protein purifn. by inactivation with inteins in specific regions and controllable intein splicing; methods for detg. crit., generalizable residues for varying intein activity.

IC ICM C12N015-57
ICS C12N015-55; C12N015-54; C12N015-62; C12N015-70; C12N009-52; C12N009-22; C12N009-10; C12P021-06; C12Q001-37; C12Q001-68

CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 6, 9, 10

ST intein fusion **protein** sepn splicing cleavage site mutagenesis

IT **Protein** motifs
(Cys rich region, intein inserted into; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT **Proteins**, specific or class
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(MBP (maltose-binding **protein**), fusion product with intein; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT **Chromatography**
(**affinity**; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and

methods for detg. crit. residues for varying cleavage activity)

IT **Protein** motifs
(endonuclease domain, deletion in inteins; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT Inteins
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion product with other **proteins**; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns.)

IT Culture media
Escherichia coli
Genetic selection
PCR (polymerase chain reaction)
Plasmid vectors
Protein engineering
Protein splicing
Structure-activity relationship
(genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT **Proteins**, specific or class
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(green fluorescent, as reporter; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT **Protein** motifs
(maltose binding domain, fusion product with intein; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT Fusion **proteins** (chimeric **proteins**)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(of intein and other **protein** or enzymes; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT Fusion **proteins** (chimeric **proteins**)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(tripartite (with a N-terminal domain, a C-terminal domain and a joining segment) for intein insertion; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns.)

IT **Protein** motifs
(zinc finger, intein inserted into; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)

IT **70-47-3**, Asparagine, biological studies 71-00-1, L-Histidine,

biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(at the C-terminus of mutant intein; genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for biosepns. and methods for detg. crit. residues for varying cleavage activity)
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 7 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:13437 HCAPLUS
DOCUMENT NUMBER: 134:338919
TITLE: Heterogeneity of glycoprotein fractions in blood serum of tumor-bearing animals
AUTHOR(S): Surgova, T. M.; Sidorenko, M. V.; Galich, I. P.; Evtushenko, N. V.
CORPORATE SOURCE: R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kiev, 03022, Ukraine
SOURCE: Eksperimental'naya Onkologiya (2000), 22(3), 148-152
CODEN: EKSODD; ISSN: 0204-3564
PUBLISHER: Institut Eksperimental'noi Patologii, Onkologii i Radiobiologii im. R. E. Kavetskogo NAN Ukrainy
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB Glycoprotein fractions (GPF) from blood serum of tumor-bearing animals (B16 melanoma and Guerin carcinoma) were purified by affinity chromatog. on Sepharose columns and analyzed. The growth of transplantable tumors was shown to be accompanied by the changes in structure and properties of GPFs under study, in particular by the changes in sugar content in the carbohydrate moieties of GPFs. The content of fucose in serum decreased as far as the tumors were increasing in size.
CC 14-1 (Mammalian Pathological Biochemistry)
IT **Proteins**, general, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(blood; heterogeneity of glycoprotein and amino acid fractions in blood serum of tumor-bearing animals)
IT **Affinity chromatography**
Blood serum
Heterogeneity
Melanoma
Transformation, neoplastic
(heterogeneity of glycoprotein and amino acid fractions in blood serum of tumor-bearing animals)
IT 56-41-7, L-Alanine, biological studies 56-45-1, L-Serine, biological studies 56-85-9, L-Glutamine, biological studies 56-86-0, L-Glutamic acid, biological studies 56-87-1, L-Lysine, biological studies 61-90-5, L-Leucine, biological studies 63-91-2, L-Phenylalanine, biological studies 70-26-8, L-Ornithine **70-47-3**, L-Asparagine, biological studies 71-00-1, L-Histidine, biological studies 72-18-4, L-Valine, biological studies 72-19-5, L-Threonine, biological studies 73-32-5, L-Isoleucine, biological studies 74-79-3, L-Arginine, biological studies 2438-80-4, L-Fucose 3416-24-8, Glucosamine
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(heterogeneity of glycoprotein and amino acid fractions in blood serum

of tumor-bearing animals)

L18 ANSWER 8 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:756857 HCAPLUS
DOCUMENT NUMBER: 133:330479
TITLE: Synthetic transmembrane peptides used to alter the properties of membrane-assocd. proteins
INVENTOR(S): Lawson, Alastair David Griffiths; Finney, Helene Margaret
PATENT ASSIGNEE(S): Celltech Therapeutics Ltd., UK
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000063374	A1	20001026	WO 2000-GB1476	20000417
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1171596	A1	20020116	EP 2000-925464	20000417
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002541845	T2	20021210	JP 2000-612453	20000417

PRIORITY APPLN. INFO.:
GB 1999-8816 A 19990416
GB 1999-8818 A 19990416
WO 2000-GB1476 W 20000417

AB The invention relates to methods of altering the properties of membrane-assocd. proteins, by substituting the transmembrane region with synthetic transmembrane peptides. In this way, for example, the level of expression of the protein can be altered as well as properties of the protein itself, such as the relative response of the membrane-assocd. protein to cell surface-assocd. antigen vs. antigen in soln. and the sensitivity of intracellular signalling mediated by the membrane-assocd. protein. The transmembrane domains may be derived from known transmembrane proteins or they may be synthetic. Synthetic peptides contg. features typical of transmembrane domains are described. The method is demonstrated with a no. of natural and synthetic transmembrane domains in a series of chimeric receptors derived from proteins of the immune system. The design of the synthetic transmembrane region allows various properties of a protein, such as its level of expression, and relative response to ligand binding to be tailored as required for a specific purpose.

IC ICM C12N015-12
ICS C07K014-705; C12N015-62; A61K031-70
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 6, 15
IT Protein motifs
(extracellular **ligand** binding domains; synthetic

transmembrane peptides used to alter properties of membrane-assocd. proteins)

IT 52-90-4, L-Cysteine, biological studies 56-41-7, L-Alanine, biological studies 56-85-9, L-Glutamine, biological studies 60-18-4, Tyrosine, biological studies 61-90-5, L-Leucine, biological studies 63-68-3, L-Methionine, biological studies 63-91-2, L-Phenylalanine, biological studies 70-47-3, L-**Asparagine**, biological studies 71-00-1, L-Histidine, biological studies 72-18-4, L-Valine, biological studies 73-22-3, L-Tryptophan, biological studies 73-32-5, Isoleucine, biological studies 147-85-3, Proline, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(in synthetic transmembrane domains; synthetic transmembrane peptides used to **alter** properties of membrane-assocd. proteins)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 9 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:548531 HCAPLUS
DOCUMENT NUMBER: 133:232973
TITLE: CCK-B/gastrin receptor transmembrane domain mutations selectively alter synthetic agonist efficacy without affecting the activity of endogenous peptides
AUTHOR(S): Blaker, Michael; Ren, Yong; Seshadri, Lakshmi; McBride, Edward W.; Beinborn, Martin; Kopin, Alan S.
CORPORATE SOURCE: Department of Medicine and the GRASP Digestive Disease Center, Tupper Research Institute, New England Medical Center, Boston, MA, USA
SOURCE: Molecular Pharmacology (2000), 58(2), 399-406
CODEN: MOPMA3; ISSN: 0026-895X
PUBLISHER: American Society for Pharmacology and Experimental Therapeutics
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recent efforts have focused on identifying small nonpeptide mols. that can mimic the activity of endogenous peptide hormones. Understanding the mol. basis of ligand-induced receptor activation by these divergent classes of ligands should expedite the process of drug development. Using the cholecystokinin-B/gastrin receptor (CCK-BR) as a model system, we have recently shown that both affinity and efficacy of nonpeptide ligands are markedly affected by amino acid alterations within a putative transmembrane domain (TMD) ligand pocket. In this report, we examine whether residues projecting into the TMD pocket det. the pharmacol. properties of structurally diverse CCK-BR ligands, including peptides and synthetic peptide-derived partial agonists (peptoids). Nineteen mutant human CCK-BRs, each including a single TMD amino acid substitution, were transiently expressed in COS-7 cells and characterized. Binding affinities as well as ligand-induced inositol phosphate prodn. at the mutant CCK-BRs were assessed for peptides (CCK-8 and CCK-4) and for peptoids (PD-135158 and PD-136450). Distinct as well as overlapping determinants of peptide and peptoid binding affinity were identified, supporting that both classes of ligands, at least in part, interact with the CCK-BR TMD ligand pocket. Eight point mutations resulted in marked increases or decreases in the functional activity of the synthetic peptoid ligands. In contrast, the functional activity of both peptides, CCK-8 and CCK-4, was not affected by any of the CCK-BR mutations. These findings suggest that the mechanisms underlying activation of G-protein-coupled receptors by endogenous peptide hormones vs. synthetic ligands may

markedly differ.
CC 2-2 (Mammalian Hormones)
Section cross-reference(s): 1
ST CCKB receptor structure activity **ligand** efficacy
IT Structure-activity relationship
(**ligand**-binding; CCK-B/gastrin receptor transmembrane domain mutations selectively alter synthetic agonist efficacy without affecting the activity of endogenous peptides)
IT 70-47-3, L-**Asparagine**, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(human CCKB receptor residue 353; CCK-B/gastrin receptor transmembrane domain mutations selectively **alter** synthetic agonist efficacy without affecting the activity of endogenous peptides)
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 10 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:410563 HCAPLUS
DOCUMENT NUMBER: 133:129990
TITLE: Multiple interactions of the Asp2.61(98) side chain of the Gonadotropin-Releasing Hormone Receptor contribute differentially to **ligand** interaction
AUTHOR(S): Flanagan, Colleen A.; Rodic, Vladimir; Konvicka, Karel; Yuen, Tony; Chi, Ling; Rivier, Jean E.; Millar, Robert P.; Weinstein, Harel; Sealfon, Stuart C.
CORPORATE SOURCE: Department of Neurology Fishberg Research Center in Neurobiology, Mount Sinai School of Medicine, New York, NY, 10029, USA
SOURCE: Biochemistry (2000), 39(28), 8133-8141
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutation of Asp2.61(98) at the extracellular boundary of transmembrane helix 2 of the gonadotropin-releasing hormone (GnRH) receptor decreased the affinity for GnRH. Using site-directed mutagenesis, ligand modification, and computational modeling, different side chain interactions of Asp2.61(98) that contribute to high-affinity binding were investigated. The conservative Asp2.61(98)Glu mutation markedly decreased the affinity for a series of GnRH analogs contg. the native His2 residue. This mutant showed smaller decreases in affinity for His2-substituted ligands. The loss of preference for His2-contg. ligands in the mutant receptor shows that Asp2.61(98) dets. the specificity for His2. Anal. of the affinities of a series of position 2-substituted ligands suggests that a hydrogen bond forms between Asp2.61(98) and the .delta. NH group of His2 and that Asp2.61(98) forms a second hydrogen bond with the ligand. Substitution of Asp2.61(98) with an uncharged residue further decreased the affinity for all ligands and also decreased receptor expression. Computational modeling indicates an intramol. ionic interaction of Asp2.61(98) with Lys3.32(121) in transmembrane helix 3. The uncharged, Lys3.32(121)Gln mutation also markedly decreased agonist affinity. The modeling and the similar phenotypes of mutants with uncharged substitutions for Asp2.61(98) or Lys3.32(121) are consistent with the presence of this helix 2-helix 3 interaction. These studies support a dual role for Asp2.61(98): formation of an interhelical interaction with Lys3.32(121) that contributes to the structure of the agonist binding pocket and an interaction with His2 of GnRH that helps stabilize agonist

complexing.
CC 2-2 (Mammalian Hormones)
ST GnRH receptor mutant asparagine histidine **ligand** binding structure activity
IT Gonadotropin-releasing hormone receptor
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); PRP (Properties); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(**asparagine** at 98 position; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand modification**, computational modeling and receptor functions)
IT Hydrogen bond
Molecular modeling
(identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)
IT Structure-activity relationship
(**ligand**-binding; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)
IT Gonadotropin-releasing hormone receptor
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); PRP (Properties); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(mutants, asparagine at 98 position mutated to Glu, Asn, Ala, and Val.; multiple interactions of the Asp2.61(98) side chain of the Gonadotropin-Releasing Hormone Receptor contribute differentially to **ligand** interaction)
IT Carboxyl group
(neg. charge of-; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)
IT Helix (conformation)
(protein; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)
IT Structure-activity relationship
(receptor-binding; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)
IT Amino group
(.delta.- , of histidine 2 side chain of GnRH and its analog; multiple interactions of the Asp2.61(98) side chain of the Gonadotropin-Releasing Hormone Receptor contribute differentially to **ligand** interaction)
IT 9034-40-6DP, GnRH, analogs
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
(identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 120287-85-6, Cetrorelix
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 38234-19-4, 1-9-Luteinizing hormone-releasing factor (swine) 39064-63-6 41621-70-9 47922-48-5, Luteinizing hormone-releasing factor I (chicken) 52435-06-0 53607-20-8 53607-21-9 91097-16-4, Luteinizing hormone-releasing factor II (chicken) 109305-49-9 286965-34-2 286965-35-3 286965-36-4 286965-37-5 286965-38-6
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 68247-19-8, Inositol phosphate
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 56-85-9, L-Glutamine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(of mutant GnRH receptor at 121 position; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 56-41-7, L-Alanine, biological studies 56-84-8, Aspartic acid, biological studies 56-86-0, L-Glutamic acid, biological studies 72-18-4, L-Valine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(of mutant GnRH receptor at 98 position; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 56-87-1, L-Lysine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(position 121 of GnRH receptor; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 71-00-1, L-Histidine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(position 2 of GnRH; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand** modification, computational modeling and receptor functions)

IT 70-47-3, L-**Asparagine**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(position 98 of GnRH receptor; identification and characterization of side chain interactions of Asp2.61(98) in GnRH receptor using mutagenesis, **ligand modification**, computational modeling and receptor functions)

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 11 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:278099 HCAPLUS
DOCUMENT NUMBER: 132:319505
TITLE: A method of **affinity separation** and immobilized **ligands** with **modified asparagine** residues for use therein
INVENTOR(S): Uhlen, Mathias; Hober, Sophia
PATENT ASSIGNEE(S): Affibody Technology Sweden AB, Swed.; Gardner, Rebecca
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023580	A1	20000427	WO 1999-GB3484	19991021
W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9963532	A1	20000508	AU 1999-63532	19991021
EP 1123389	A1	20010816	EP 1999-950942	19991021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, IE, SI, LT, LV, FI, RO				
JP 2002527107	T2	20020827	JP 2000-577291	19991021
NO 2001001969	A	20010619	NO 2001-1969	20010420

PRIORITY APPLN. INFO.: GB 1998-23071 A 19981021
WO 1999-GB3484 W 19991021

AB The present invention relates to methods of affinity sepn. wherein the affinity ligand is an immobilized proteinaceous ligand wherein one or more of its asparagine (Asn) residues has been modified. Methods of making a stabilized combinatorial protein comprising the steps of: (a) modification of Asn residues within a protein mol. to increase stability of the protein in alk. conditions; and (b) randomization of the protein mol. to modify its binding characteristics and combinatorial proteins wherein in a step sep. from the randomization step, the stability of the protein in alk. conditions has been increased by modifying one or more of its Asn residues are also described. Alk. resistant mutant albumin-binding proteins were prepd. by cloning and PCR mutagenesis. Various Asn residues were changed to more stable residues. Mutant ABDmut was coupled to carbodiimide CMC to make an affinity column for purifn. of human serum albumin.

IC ICM C12N015-10
ICS C07K001-22; C07K014-31

CC 9-3 (Biochemical Methods)
Section cross-reference(s): 3
ST **affinity sepn** immobilized **protein** **modified asparagine; alk resistant** mutant albumin binding **protein;** serum albumin **affinity chromatog** column
IT **Proteins,** specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(A, staphylococcal, combinatorial **protein** derived from; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT **Proteins,** specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(DNA-binding, combinatorial **protein** derived from; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT **Separation**
(**affinity;** method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT **Proteins,** specific or class
RL: DEV (Device component use); NUU (Other use, unclassified); PRP (Properties); USES (Uses)
(albumin-binding, with **modified asparagines;** method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT Mutagenesis
(by PCR; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT Immunoglobulins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(combinatorial **protein** derived from; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT **Protein** motifs
(domain Z; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT Cell
(expressing **protein** with **modified asparagines;** method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT Gene
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(for **protein** with **modified asparagines;** method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)
IT **Ligands**
RL: DEV (Device component use); NUU (Other use, unclassified); PRP (Properties); USES (Uses)

(immobilized **protein**; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT **Proteins**, specific or class
RL: DEV (Device component use); NUU (Other use, unclassified); PRP (Properties); USES (Uses)
(immobilized, with **modified asparagines**; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT **Affinity chromatography**
Molecular cloning
Phage display
Phage display library
(method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT PCR (polymerase chain reaction)
(mutagenesis; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT DNA sequences
(of albumin binding **protein** mutants; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT Combinatorial library
(of **proteins** with **modified asparagines** for stability in alk. conditions; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT Albumins, analysis
RL: ANT (Analyte); PUR (Purification or recovery); ANST (Analytical study); PREP (Preparation)
(serum; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT Fusion **proteins** (chimeric **proteins**)
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(specific binding; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 266309-43-7P
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
(amino acid sequence, as **alk. resistant** mutant; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 56-84-8, Aspartic acid, properties 56-87-1, L-Lysine, properties 61-90-5, Leucine, properties
RL: PRP (Properties)
(**asparagine** residues replacement with; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 15580-20-8DP, conjugates with albumin binding **proteins**
RL: DEV (Device component use); NUU (Other use, unclassified); SPN (Synthetic preparation); PREP (Preparation); USES (Uses)

(method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 1310-73-2, Sodium hydroxide, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**modified protein** stability after treatment with; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 266299-57-4P 266299-58-5P 266299-59-6P 266299-60-9P 266299-61-0P
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
(nucleotide sequence; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT **70-47-3D, Asparagine, modified,** properties
RL: PRP (Properties)
(of **protein ligand;** method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 266299-77-8, 2: PN: WO0023580 SEQID: 8 unclaimed DNA 266299-78-9, 3: PN: WO0023580 SEQID: 9 unclaimed DNA 266299-79-0, 4: PN: WO0023580 SEQID: 10 unclaimed DNA 266299-80-3, 10: PN: WO0023580 SEQID: 2 unclaimed DNA 266299-81-4, 11: PN: WO0023580 SEQID: 3 unclaimed DNA 266299-82-5, 12: PN: WO0023580 SEQID: 4 unclaimed DNA 266299-83-6, 13: PN: WO0023580 SEQID: 5 unclaimed DNA 266299-84-7, 18: PN: WO0023580 SEQID: 6 unclaimed DNA 266299-85-8, 19: PN: WO0023580 SEQID: 7 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

IT 266309-36-8 266309-42-6
RL: PRP (Properties)
(unclaimed **protein** sequence; method of **affinity sepn.** and immobilized **ligands** with **modified asparagine** residues for use therein)

L18 ANSWER 12 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:341262 HCAPLUS
DOCUMENT NUMBER: 131:99386
TITLE: Serial lectin **affinity chromatography** demonstrates **altered asparagine**-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma
AUTHOR(S): Sumi, Shuhei; Arai, Kyoko; Kitahara, Satoshi; Yoshida, Ken-Ichiro
CORPORATE SOURCE: Department of Urology, Dokkyo University School of Medicine, Tochigi, 321-0293, Japan
SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1999), 727(1 + 2), 9-14
CODEN: JCBBEP; ISSN: 0378-4347
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Differences between prostate carcinoma (PCA) and benign prostatic hyperplasia (BPH) in asparagine (N)-linked sugar-chain structures of prostate-specific antigen (PSA) were investigated using serial lectin

affinity chromatog. The amts. of PSA passing through columns of Con A, phytohemagglutinin E4 (PHA-E4) and PHA-L4 were significantly greater for PCA-derived PSA than BPH. We propose that the sugar moiety structure of PSA which is increased in PCA is a multiantennary complex type with branched N-acetylglucosamine .beta.(1.fwdarw.4) mannose. We suggest that N-linked sugar chains in PSA are altered during oncogenesis in the human prostate and may serve as diagnostic tools for PCA.

CC 9-3 (Biochemical Methods)
Section cross-reference(s): 14

ST lectin **affinity chromatog** prostate specific antigen structure carcinoma

IT Prostate gland
(benign hyperplasia; serial lectin **affinity chromatog**. demonstrates **altered asparagine**-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma)

IT Prostate gland
(carcinoma; serial lectin **affinity chromatog**. demonstrates **altered asparagine**-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma)

IT **Affinity chromatography**
(serial lectin **affinity chromatog**. demonstrates **altered asparagine**-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma)

IT Prostate-specific antigen
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(serial lectin **affinity chromatog**. demonstrates **altered asparagine**-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma)

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 13 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:194262 HCAPLUS
DOCUMENT NUMBER: 130:232846
TITLE: Analogs of human Mpl **ligand**/thrombopoietin analogs with modified glycosylation sites
INVENTOR(S): Elliott, Steven G.
PATENT ASSIGNEE(S): Amgen Inc., USA
SOURCE: PCT Int. Appl., 84 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9913076	A1	19990318	WO 1998-US18753	19980909

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 5989538	A	19991123	US 1997-927855	19970911
CA 2303480	AA	19990318	CA 1998-2303480	19980909
AU 9893103	A1	19990329	AU 1998-93103	19980909
AU 727978	B2	20010104		
EP 1012284	A1	20000628	EP 1998-945978	19980909
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001515719	T2	20010925	JP 2000-510861	19980909
MX 200002467	A	20001030	MX 2000-2467	20000310

PRIORITY APPLN. INFO.:

US 1997-927855	A	19970911
US 1995-388779	A2	19950215
US 1995-451086	A3	19950525
US 1996-591070	A2	19960209
WO 1998-US18753	W	19980909

AB Analogs of human mpl ligand (thrombopoietin) having .gtoreq.1 changed glycosylation sites as compared to a naturally occurring mpl ligand sequence of a corresponding no. of amino acids are disclosed. The invention also relates to DNA sequences encoding said mpl ligand analogs, recombinant plasmids and host cells for analog expression, and therapeutic compns. including such analogs. The analogs can activate the mpl receptor and thus enhance megakaryocytopoiesis, that results in an increase in platelets in vivo. Prepn. of [1-174]-mpl ligand and its substitution mutants in transgenic CHO cells was shown and their biol. activities characterized.

IC ICM C12N015-12
ICS C12N005-10; C07K014-52; A61K038-19

CC 2-2 (Mammalian Hormones)
Section cross-reference(s): 3

ST Mpl **ligand** glycosylation site analog; thrombopoietin glycosylation site analog; sequence thrombopoietin analog human

IT Animal cell line
(CHO, cloning host; analogs of human Mpl **ligand** /thrombopoietin analogs with modified glycosylation sites)

IT Animal cell line
(COS, cloning host; analogs of human Mpl **ligand** /thrombopoietin analogs with modified glycosylation sites)

IT Molecular cloning
Platelet (blood)
Protein engineering
(analogs of human Mpl **ligand**/thrombopoietin analogs with modified glycosylation sites)

IT Escherichia coli
(cloning host; analogs of human Mpl **ligand**/thrombopoietin analogs with modified glycosylation sites)

IT Protein motifs
(glycosylation site; analogs of human Mpl **ligand** /thrombopoietin analogs with modified glycosylation sites)

IT Animal cell
(mammalian, cloning host; analogs of human Mpl **ligand** /thrombopoietin analogs with modified glycosylation sites)

IT Hematopoiesis
(megakaryocytopoiesis; analogs of human Mpl **ligand** /thrombopoietin analogs with modified glycosylation sites)

IT Protein sequences
(of analogs of human Mpl **ligand**/thrombopoietin analogs with modified glycosylation sites)

IT 162002-59-7DP, Thrombopoietin (human), analogs 168181-00-8DP, 7-174-Thrombopoietin (human), analogs 168181-21-3P, 1-174-Thrombopoietin

(human) 168181-22-4P, 1-191-Thrombopoietin (human) 169442-42-6DP, 7-332-Thrombopoietin (human), analogs 182238-51-3P, 1-174-Thrombopoietin [22-**asparagine**] (human) 182238-52-4P, 1-174-Thrombopoietin [25-**asparagine**] (human) 182238-53-5P, 1-174-Thrombopoietin [30-**asparagine**,32-threonine] (human) 182238-54-6P, 1-174-Thrombopoietin [38-**asparagine**,40-threonine] (human) 182238-55-7P, 1-174-Thrombopoietin [86-**asparagine**] (human) 182238-56-8P, 1-174-Thrombopoietin [82-**asparagine**,83-alanine] (human) 182238-57-9P, 1-174-Thrombopoietin [87-**asparagine**,89-threonine] (human) 182238-58-0P, 1-174-Thrombopoietin [92-**asparagine**] (human) 182238-59-1P, 1-174-Thrombopoietin [120-**asparagine**,122-threonine] (human) 182238-60-4P, 1-174-Thrombopoietin [36-serine,38-**asparagine**,40-threonine] (human) 182238-61-5P, 1-174-Thrombopoietin [88-**asparagine**,90-threonine] (human) 182238-62-6P, 1-174-Thrombopoietin [53-**asparagine**,55-threonine] (human) 182238-63-7P, 1-174-Thrombopoietin [58-**asparagine**,60-threonine] (human) 182238-64-8P, 1-174-Thrombopoietin [30-**asparagine**,32-threonine,120-**asparagine**,122-threonine] (human) 182238-65-9P, 1-183-Thrombopoietin (human) 182238-66-0DP, 7-191-Thrombopoietin (human), analogs 182238-67-1DP, 7-199-Thrombopoietin (human), analogs 182238-68-2DP, 7-183-Thrombopoietin (human), analogs 182238-69-3P, 1-174-Thrombopoietin [23-**asparagine**,25-serine] (human) 182238-70-6P, 1-174-Thrombopoietin [37-**asparagine**,38-glycine,39-serine] (human) 182238-71-7P, 1-174-Thrombopoietin [39-**asparagine**,41-serine] (human) 182238-72-8P, 1-174-Thrombopoietin [54-**asparagine**,56-serine] (human) 182238-73-9P, 1-174-Thrombopoietin [52-**asparagine**,54-threonine] (human) 182238-74-0P, 1-174-Thrombopoietin [55a-endo-**asparagine**,57-threonine] (human) 182238-75-1P, 1-174-Thrombopoietin [57-**asparagine**,59-serine] (human) 182238-76-2P, 1-174-Thrombopoietin [81-**asparagine**,83-threonine] (human) 182238-77-3P, 1-174-Thrombopoietin [118-**asparagine**,120-serine] (human) 182238-78-4P, 1-174-Thrombopoietin [119-**asparagine**,121-serine] (human) 182238-79-5P, 1-174-Thrombopoietin [30-**asparagine**,32-serine,120-**asparagine**,122-serine] (human) 182238-80-8P, 1-174-Thrombopoietin [163-threonine,164-**asparagine**] (human) 182238-81-9P, 1-174-Thrombopoietin [30-**asparagine**,32-threonine,endo-55a-**asparagine**,57-threonine,120-**asparagine**,122-threonine] (human) 182238-83-1P, 1-174-Thrombopoietin [55-**asparagine**,57-threonine] (human) 182238-84-2P, 1-174-Thrombopoietin [56-**asparagine**] (human) 182238-85-3P, 1-174-Thrombopoietin [163-threonine,164-**asparagine**,166-threonine] (human) 182298-88-0P, 1-199-Thrombopoietin (human) 221183-52-4P 221183-53-5P 221183-57-9P 221183-58-0P 221183-59-1P 221183-60-4P 221183-61-5P
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(amino acid sequence; analogs of human Mpl **ligand**/thrombopoietin analogs with **modified** glycosylation sites)

IT 9014-42-0DP, Thrombopoietin, analogs
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(analogs of human Mpl **ligand**/thrombopoietin analogs with modified glycosylation sites)

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 14 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:167338 HCAPLUS
DOCUMENT NUMBER: 130:348866
TITLE: **Alterations** of **asparagine**-linked sugar chains of N-acetyl .beta.-D-hexosaminidase during human renal oncogenesis: a preliminary study using serial lectin **affinity chromatography**
AUTHOR(S): Yoshida, Ken-ichiro; Moriguchi, Hideo; Sumi, Shuhei; Horimi, Hiroyuki; Kitahara, Satoshi; Umeda, Hiroshi; Ueda, Yoshihiko
CORPORATE SOURCE: Departments of Urology and Pathology, Dokkyo University School of Medicine, Shimotsuga-gun, Mibu-machi, Tochigi, 321-0293, Japan
SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1999), 723(1 + 2), 75-80
CODEN: JCBBEP; ISSN: 0378-4347
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymic properties and asparagine (Asn)-linked sugar-chain structures of N-acetyl .beta.-D-hexosaminidase A (Hex A) were compared in human tissues between normal renal cortex and renal cell carcinoma (RCC). No significant differences between the two Hex A prepns. were obsd. with respect to enzymic properties such as mol. mass, Michaelis-Menten value or optimal pH. With RCC prepns., relatively more Hex A passed through the Con A column, bound weakly to Con A, or bound strongly to Con A and also to the wheat germ agglutinin (WGA) column, than with prepns. from normal renal cortex. In contrast, relatively less Hex A bound strongly to the Con A column, but passed through the WGA column with RCC prepns. than with those from normal renal cortex. Asn-linked sugar-chain structures might apparently be altered during human renal oncogenesis.

CC 7-2 (Enzymes)
Section cross-reference(s): 13, 14

IT Oligosaccharides, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation)
(branched; **alterations** of **asparagine**-linked sugar chains of N-acetyl .beta.-D-hexosaminidase during human renal oncogenesis)

IT Kidney
(cortex; **alterations** of **asparagine**-linked sugar chains of N-acetyl .beta.-D-hexosaminidase during human renal oncogenesis)

IT Kidney, neoplasm
(renal cell carcinoma; **alterations** of **asparagine**-linked sugar chains of N-acetyl .beta.-D-hexosaminidase during human renal oncogenesis)

IT 9012-33-3P, N-Acetyl .beta.-D-hexosaminidase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(**alterations** of **asparagine**-linked sugar chains of N-acetyl .beta.-D-hexosaminidase during human renal oncogenesis)

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 15 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:6488 HCAPLUS
DOCUMENT NUMBER: 128:72322
TITLE: Histidine .fwdarw. Carboxamide **Ligand** Substitutions in the Zinc Binding Site of Carbonic Anhydrase II Alter Metal Coordination Geometry but Retain Catalytic Activity
AUTHOR(S): Lesburg, Charles A.; Huang, Chih-chin; Christianson, David W.; Fierke, Carol A.
CORPORATE SOURCE: Department of Chemistry, University of Pennsylvania, Philadelphia, PA, 19104-6323, USA
SOURCE: Biochemistry (1997), 36(50), 15780-15791
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The catalytic zinc ion of human carbonic anhydrase II (CAII) is coordinated by three histidine ligands (H94, H96, and H119) and a hydroxide ion with tetrahedral geometry. Structural and functional anal. of variants in which the zinc ligands H94 and H119 are substituted with asparagine and glutamine, and comparison with results obtained with aspartate and glutamate substitutions indicate that the neutral ligand field provided by the protein optimizes the electrostatic environment for the catalytic function of the metal ion, including stabilization of bound anions. This is demonstrated by catalytic activity measurements for ester hydrolysis and CO2 hydration, as well as sulfonamide inhibitor affinity assays. High-resoln. X-ray crystal structure detns. of H94N, H119N, and H119Q CAIIs reveal that the engineered carboxamide side chains coordinate to zinc with optimal stereochem. However, zinc coordination geometry remains tetrahedral only in H119Q CAII. Metal geometry changes to trigonal bipyramidal in H119N CAII due to the addn. of a second water mol. to the zinc coordination polyhedron and also in H94N CAII due to the displacement of zinc-bound hydroxide by the bidentate coordination of a Tris mol. Possibly, the bulky histidine imidazole ligands of the native enzyme play a role in disfavoring trigonal bipyramidal coordination geometry for zinc. Protein-metal affinity is significantly compromised by all histidine .fwdarw. carboxamide ligand substitutions. Diminished affinity may result from significant movements (up to 1 .ANG.) of the metal ion from its position in the wild-type enzyme, as well as the assocd., minor conformational changes of metal ligands and their neighboring residues.

CC 7-5 (Enzymes)
Section cross-reference(s): 75

ST histidine carboxamide zinc **ligand** carbonic anhydrase; coordination zinc carbonic anhydrase histidine carboxamide; crystal structure carbonic anhydrase variant

IT Enzyme functional sites
(active; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT Crystal structure
Enzyme kinetics
(histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT Enzyme functional sites
(metal-binding; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT Dissociation constant
Dissociation kinetics
(of zinc; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT Coordination (structure)
(zinc coordination geometry; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT 9001-03-0, Carbonic anhydrase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(II; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT 7440-66-6, Zinc, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT 56-85-9, L-Glutamine, biological studies 70-47-3, L-**Asparagine**, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(replacement of zinc **ligand** histidine residue; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II **alter** metal coordination geometry but retain catalytic activity)

IT 59-66-5, Acetazolamide 1431-39-6, Dansylamide
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(sulfonamide inhibitor binding; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT 71-00-1, L-Histidine, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(zinc **ligand** residues 94 and 119; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

IT 14280-30-9, Hydroxide, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(zinc **ligand**; histidine .fwdarw. carboxamide **ligand** substitutions in the zinc binding site of carbonic anhydrase II alter metal coordination geometry but retain catalytic activity)

L18 ANSWER 16 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:469688 HCAPLUS
DOCUMENT NUMBER: 127:203652
TITLE: Serial lectin **affinity chromatography** with concanavalin A and wheat germ agglutinin demonstrates **altered asparagine**-linked sugar-chain structures of prostatic acid phosphatase in human prostate carcinoma
AUTHOR(S): Yoshida, Ken-ichiro; Honda, Mikihiko; Arai, Kyoko; Hosoya, Yoshakatsu; Moriguchi, Hideo; Sumi, Shuhei; Ueda, Yoshihiko; Kitahara, Satoshi
CORPORATE SOURCE: Department of Urology, Dokkyo University School of Medicine, Mibu, Tochigi, Japan
SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1997), 695(2), 439-443
CODEN: JCBBEP; ISSN: 0378-4347
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Differences between human prostate carcinoma (PCA, 5 cases) and benign prostatic hyperplasia (BPH, 5 cases) in asparagine-linked (Asn) sugar-chain structure of prostatic acid phosphatase (PAP) were investigated using lectin affinity chromatog. with Con A and wheat germ agglutinin (WGA). PAP activities were significantly decreased in PCA-derived PAP, whereas no significant differences between the two PAP prepns. were obsd. in the enzymic properties (Michaelis-Menten value, optimal pH, thermal stability, and inhibition study). In these PAP prepns., all activities were found only in the fractions which bound strongly to the Con A column and were undetectable in the Con A unbound fractions and in the fractions which bound weakly to the Con A column. The relative amts. of PAP which bound strongly to the Con A column but passed through the WGA column, were significantly greater in BPH-derived PAP than in PCA-derived PAP. In contrast, the relative amts. of PAP which bound strongly to the Con A column and bound to the WGA column, were significantly greater in PCA-derived PAP than in BPH-derived PAP. The findings suggest that Asn-linked sugar-chain structures are altered during oncogenesis in human prostate and also suggest that studies of qual. differences of sugar-chain structures of PAP might lead to a useful diagnostic tool for PCA.

CC 14-1 (Mammalian Pathological Biochemistry)
Section cross-reference(s): 7

IT Oligosaccharides, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(**asparagine**-linked; serial lectin **affinity chromatog.** with Con A and wheat germ agglutinin demonstrates **altered asparagine**-linked sugar-chain structures of prostatic acid phosphatase in human prostate carcinoma)

IT Prostate gland
(benign hyperplasia; serial lectin **affinity chromatog**. with Con A and wheat germ agglutinin demonstrates **altered asparagine**-linked sugar-chain structures of prostatic acid phosphatase in human prostate carcinoma)

IT Prostate gland
(carcinoma; serial lectin **affinity chromatog.** with Con A and wheat germ agglutinin demonstrates **altered asparagine**-linked sugar-chain structures of prostatic acid

phosphatase in human prostate carcinoma)
IT 9001-77-8, Acid phosphatase
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(serial lectin **affinity chromatog.** with Con A and wheat germ agglutinin demonstrates **altered asparagine** -linked sugar-chain structures of prostatic acid phosphatase in human prostate carcinoma)

L18 ANSWER 17 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:195943 HCAPLUS
DOCUMENT NUMBER: 124:252599
TITLE: Primer-terminus stabilization at the 3'-5' exonuclease active site of .vphi.29 DNA polymerase. Involvement of two amino acid residues highly conserved in proofreading DNA polymerases
AUTHOR(S): de Vega, Miguel; Lazaro, Jose M.; Salas, Margarita; Blanco, Luis
CORPORATE SOURCE: Cent. Biol. Mol. "Severo Ochoa', Univ. Autonoma, Madrid, 28049, Spain
SOURCE: EMBO Journal (1996), 15(5), 1182-92
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB By site-directed mutagenesis in .vphi.29 DNA polymerase, we have analyzed the functional importance of two evolutionarily conserved residues belonging to the 3'-5' exonuclease domain of DNA-dependent DNA polymerases. In Escherichia coli DNA polymerase I, these residues are Thr358 and Asn420, shown by crystallog. anal. to be directly acting as single-stranded DNA (ssDNA) ligands at the 3'-5' exonuclease active site. On the basis of these structural data, single substitution of the corresponding residues of .vphi.29 DNA polymerase, Thr15 and Asn62, produced enzymes with a very reduced or altered capacity to bind ssDNA. Anal. of the residual 3'-5' exonuclease activity of these mutant derivs. on ssDNA substrates allowed us to conclude that these two residues do not play a direct role in the catalysis of the reaction. On the other hand, anal. of the 3'-5' exonuclease activity on either matched or mismatched primer/template structures showed a crit. role of these two highly conserved residues in exonucleolysis under polymn. conditions, i.e. in the proofreading of DNA polymn. errors, an evolutionary advantage of most DNA-dependent DNA polymerases. Moreover, in contrast to the dual role in 3'-5' exonucleolysis and strand displacement previously obsd. for .vphi.29 DNA polymerase residues acting as metal ligands, the contribution of residues Thr15 and Asn62 appears to be restricted to the proofreading function, by stabilization of the frayed primer-terminus at the 3'-5' exonuclease active site.
CC 3-4 (Biochemical Genetics)
Section cross-reference(s): 7, 10
IT Deoxyribonucleic acids
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(single-stranded; in Escherichia coli DNA polymerase I, highly conserved residues in proofreading are Thr358 and Asn420, shown by crystallog. anal. to be directly acting as single-stranded DNA **ligands** at the 3'-5' exonuclease active site)
IT 70-47-3, **Asparagine**, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological

study, unclassified); BIOL (Biological study)
(Asn62; single substitution of the corresponding residues of .vphi.29 DNA polymerase, Thr15 and Asn62, produced enzymes with a very reduced or **altered** capacity to bind ssDNA)

L18 ANSWER 18 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:865430 HCAPLUS
DOCUMENT NUMBER: 123:309143
TITLE: Serial lectin **affinity chromatography** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma
AUTHOR(S): Yoshida, Ken-ichiro; Sumi, Shuhei; Honda, Mikihiko; Hosoya, Yoshikatsu; Yano, Masataka; Arai, Kyoko; Ueda, Yoshihiko
CORPORATE SOURCE: Department of Urology, Dokkyo University School of Medicine, 880 Kobayashi, Mibu-cho, Shimotsuga-gun, Tochigi, 321-02, Japan
SOURCE: Journal of Chromatography, B: Biomedical Applications (1995), 672(1), 45-51
CODEN: JCBBEP; ISSN: 0378-4347
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Differences between human renal cortex and human renal cell carcinoma (RCC) in asparagine (Asn)-linked sugar chain structures of .gamma.-glutamyltransferase (GGT) were investigated by using a serial lectin affinity chromatog. technique. The relative amts. of GGT which passed through the Con A column but bound to the phytohemagglutinin E column, were significantly decreased in RCC, but there were significant increases in the relative amts. of GGT which bound weakly to the Con A column and passed through the pea lectin (PSA) column, and bound strongly to the Con A column and bound to the wheat germ agglutinin column in RCC compared with those of the normal renal cortex. A significant correlation was obsd. in RCC between nuclear grade and relative amt. of GGT which bound weakly to the Con A column and passed through the PSA column. The findings indicate that Asn-linked sugar chain structures are altered in RCC and suggest that studies of qual. differences of sugar chain structures of GGT might lead to a useful diagnostic tool for human RCC.

CC 7-1 (Enzymes)
Section cross-reference(s): 14
ST lectin **affinity chromatog** asparagine linked sugar; glutamyltransferase kidney cell carcinoma
IT Agglutinins and Lectins
RL: NUU (Other use, unclassified); USES (Uses)
(serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)
IT Agglutinins and Lectins
RL: NUU (Other use, unclassified); USES (Uses)
(phytohemagglutinins, serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)
IT Kidney, neoplasm
(renal cell carcinoma, serial lectin **affinity chromatog.** demonstrates **altered asparagine**

-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)

IT 57-50-1, Sugar, uses
RL: NUU (Other use, unclassified); USES (Uses)
(**asparagine**-linked; serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)

IT 9046-27-9, .gamma.-Glutamyl transferase
RL: ANT (Analyte); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)

IT 11028-71-0, Concanavalin A
RL: NUU (Other use, unclassified); USES (Uses)
(serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)

IT 70-47-3, **Asparagine**, properties
RL: PRP (Properties)
(serial lectin **affinity chromatog.** demonstrates **altered asparagine**-linked sugar chain structures of .gamma.-glutamyltransferase in human renal cell carcinoma)

L18 ANSWER 19 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:164746 HCAPLUS
DOCUMENT NUMBER: 118:164746
TITLE: Method for blocking **protein** membrane
INVENTOR(S): Kuzuhara, Akio
PATENT ASSIGNEE(S): Kanebo, Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04360900	A2	19921214	JP 1991-163570	19910606

PRIORITY APPLN. INFO.: JP 1991-163570 19910606

AB A protein membrane with immobilized antigen, antibody, enzyme, or bacteria, useful as an immunoassay reagent for diagnosis or in purifn. and sepn. of bioproducts, is blocked by covalent bonding with a protein. Preferably, the protein membrane is a silk fibroin membrane with an inclusion-immobilized antigen or antibody. The blocking method inhibits nonspecific adsorption to the protein membrane, increases the sensitivity of the protein membrane as an immunosensor, or improves the accuracy of the protein membrane as a stationary phase in affinity chromatog. Silk fibroin immobilized with anti-human .alpha.-fetoprotein mouse monoclonal antibody (prepn. given) was dipped in an aq. 1.0 wt.% polyglutaraldehyde soln. (prepn. given) at room temp. for 180.degree., thoroughly washed with 0.01 M physiol. saline to remove unreacted polyglutaraldehyde, then dipped for blocking in an 0.01-1 M soln. of an amino acid such as lysine, glycine, alanine, serine, arginine, asparagine, or aspartic acid or a 0.5 wt.% soln. of bovine serum or egg albumin in 0.1 M phosphate buffer, thoroughly washed with 0.01 M physiol. saline, and stored in a 0.1 wt.%

soln. of NaN3 in 0.01 M physiol. saline.
IC ICM C07K015-20
ICS C07K017-02; C12N011-00; G01N027-327; G01N033-543
CC 9-14 (Biochemical Methods)
Section cross-reference(s): 15, 16
ST **protein** membrane covalent blocking; silk fibroin membrane antibody immobilized; antigen immobilized **protein** membrane; enzyme immobilized **protein** membrane; bacteria immobilized **protein** membrane; immunosensor silk fibroin immobilized antibody; **affinity chromatog protein** membrane
IT **Proteins**, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(blocking by, of **protein** membrane with immobilized antigen or antibody or other agent)
IT Bacteria
Antigens
RL: ANST (Analytical study)
(immobilized, on **protein** membrane, **protein** covalent blocking of)
IT Immunoassay
(**protein** membrane with immobilized antigen or antibody for, **protein** covalent blocking of)
IT Membranes
(**protein**, with immobilized agent, covalent blocking of, with **protein**)
IT Antibodies
RL: SPN (Synthetic preparation); PREP (Preparation)
(silk fibroin membrane-immobilized, prepn. of, for immunosensors and **affinity chromatog.** stationary phases)
IT Biosensors
(with covalently blocked **protein** membrane contg. immobilized antigen or antibody or other agent)
IT **Chromatography**, column and liquid
(**affinity**, stationary phases for, covalently blocked **protein** membrane contg. immobilized antigen or antibody or other agent for)
IT Enzymes
RL: ANST (Analytical study)
(immobilized, on **protein** membrane, **protein** covalent blocking of)
IT 56-40-6, Glycine, reactions 56-41-7, Alanine, reactions 56-45-1, Serine, reactions 56-84-8, Aspartic acid, reactions 56-87-1, Lysine, reactions **70-47-3**, Asparagine, reactions 74-79-3, Arginine, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(blocking by, of silk fibroin membrane with immobilized anti-.alpha.-fetoprotein mouse monoclonal antibody)

L18 ANSWER 20 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1992:75334 HCAPLUS
DOCUMENT NUMBER: 116:75334
TITLE: Synthesis and properties of a chiral chelating resin supported by phenol-formaldehyde copolymer
AUTHOR(S): Ma, Jianbiao; He, Binglin
CORPORATE SOURCE: Inst. Polym. Chem., Nankai Univ., Tianjin, 300071, Peop. Rep. China
SOURCE: Gaodeng Xuexiao Huaxue Xuebao (1991), 12(3), 421-2
CODEN: KTHPDM; ISSN: 0251-0790

DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The microspheric phenol-formaldehyde copolymer, prepd. by suspension polymn. from phenol and formaldehyde, was grafted by L-proline through 2-hydroxy propylene spacer to give a chiral chelating resin. Its copper(II) chelate could be used as the stationary phase for the ligand-exchange chromatog. to resolve the racemates of proline, valine, isoleucine, methionine, histidine, asparagine, aspartic acid, and glutamic acid.
CC 80-4 (Organic Analytical Chemistry)
ST proline modified phenol formaldehyde copolymer phase; copper chelated proline modified stationary phase; amino acid resoln **ligand** exchange chromatog
IT Amino acids, analysis
RL: ANST (Analytical study)
(resoln. of, by **ligand**-exchange chromatog. on copper chelated proline modified phenol-formaldehyde copolymer stationary phase)
IT Chromatography, column and liquid
(**ligand**-exchange, stationary phases, copper chelated proline modified phenol-formaldehyde copolymer as, for resoln. of amino acids)
IT 59-51-8, DL-Methionine 443-79-8, DL-Isoleucine 516-06-3, DL-Valine 609-36-9, DL-Proline 617-45-8, DL-Aspartic acid 617-65-2, DL-Glutamic acid 3130-87-8, DL-**Asparagine** 4998-57-6, DL-Histidine
RL: ANST (Analytical study); PROC (Process)
(resoln. of, by **ligand**-exchange chromatog. on copper chelated proline **modified** phenol-formaldehyde copolymer stationary phase)
IT 56-84-8, L-Aspartic acid, analysis 56-86-0, L-Glutamic acid, analysis 63-68-3, L-Methionine, analysis 70-47-3, L-**Asparagine**, analysis 71-00-1, L-Histidine, analysis 72-18-4, L-Valine, analysis 73-32-5, L-Isoleucine, analysis 147-85-3, L-Proline, analysis 319-78-8, D-Isoleucine 344-25-2, D-Proline 348-67-4, D-Methionine 351-50-8, D-Histidine 640-68-6, D-Valine 1783-96-6, D-Aspartic acid 2058-58-4, D-**Asparagine** 6893-26-1, D-Glutamic acid
RL: ANST (Analytical study)
(sepn. of, from enantiomer by **ligand**-exchange chromatog. on copper chelated proline **modified** phenol-formaldehyde copolymer stationary phase)
IT 106-89-8D, reaction product with proline and phenol-formaldehyde copolymer, copper complex 147-85-3D, L-Proline, reaction product with phenol-formaldehyde copolymer and epichlorohydrin, copper complex 7440-50-8D, Copper, complex with proline modified phenol-formaldehyde copolymer 9003-35-4D, Phenol-formaldehyde copolymer, reaction product with proline and epichlorohydrin, copper complex
RL: ANST (Analytical study)
(stationary phase in resoln. of amino acids by **ligand** -exchange chromatog.)

L18 ANSWER 21 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1991:38583 HCAPLUS
DOCUMENT NUMBER: 114:38583
TITLE: Immobilized metal ion **affinity chromatography**: effect of solute structure, **ligand** density and salt concentration on the retention of peptides
AUTHOR(S): Belew, Makonnen; Porath, Jerker
CORPORATE SOURCE: Inst. Biochem., Uppsala Univ., Uppsala, S-751 23, Swed.

SOURCE: Journal of Chromatography (1990), 516(2), 333-54
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The adsorption characteristics of a variety of synthetic peptide hormones and di-, tri-, and tetrapeptides on Cu(II) immobilized on two com. available high-performance chelating gels run under various exptl. conditions are described. Methods for detg. the concn. of immobilized Cu(II) in situ are also described. The Cu(II)-charged columns exhibit a net neg. charge as judged from the significantly higher retention of some basic peptides in the absence of NaCl in the equilibration and elution buffers. At higher NaCl concns. (2-4M), arom. interactions seem to be superimposed on the metal ion affinity characteristics of the peptides. The relationship between resoln. of peptides and the concn. of immobilized Cu(II) ions has also been established for the Chelating Superose gel where 40 .mu.mol Cu(II)/mL gel apparently gives the optimum resoln. The nature of the gel matrix also plays a role in the resoln. of some peptides, the extent of which is difficult to predict. The results obtained also suggest that peptides contg. arom. and hydroxy amino acids are retarded more than those which lack them. Moreover, these same amino acids apparently strengthen the existing strong binding of peptides contg. His, Trp, or Cys to a Chelating Superose-Cu(II) column. Dipeptides with C-terminal His (i.e., X-His) are neither bound nor retarded on a column of Chelating Superose-Cu(II) whereas those having the structure His-X are strongly bound. Some tri- and tetrapeptides contg. His were also found not to bind to the column. The underlying cause of this anomalous adsorption behavior is discussed and is ascribed to metal ion transfer arising from the relatively higher affinity of such peptides towards immobilized Cu(II) ions than the chelator groups (iminodiacetate) which covalently bound to the gel matrix.

CC 9-3 (Biochemical Methods)
Section cross-reference(s): 2, 34, 66, 80
ST immobilized metal ion **affinity** HPLC; liq **chromatog** peptide; solute structure HPLC peptide; **ligand** density HPLC peptide; salt concn HPLC peptide

IT Peptides, analysis
RL: ANT (Analyte); ANST (Analytical study)
(**chromatog**. of, immobilized metal ion **affinity**)

IT **Ligands**
RL: ANST (Analytical study)
(immobilized metal ion **affinity chromatog**. response to)

IT Hormones
RL: PROC (Process)
(peptide, immobilized metal ion **affinity chromatog**. of)

IT Peptides, analysis
RL: ANT (Analyte); ANST (Analytical study)
(di-, **chromatog**. of, immobilized metal ion **affinity**)

IT **Chromatography**, column and liquid
(high-performance, **affinity**, immobilized metal ion, of peptides)

IT Peptides, analysis
RL: ANT (Analyte); ANST (Analytical study)
(tetra-, **chromatog**. of, immobilized metal ion **affinity**)

IT Peptides, analysis

RL: ANT (Analyte); ANST (Analytical study)
(tri-, **chromatog.** of, immobilized metal ion **affinity**
)

IT 50-56-6, Oxytocin, analysis 51-45-6, Histamine, analysis 51-67-2, Tyramine 52-90-4, L-Cysteine, analysis 52-90-4D, Cysteine, peptides contg. 56-40-6, Glycine, analysis 56-41-7, L-Alanine, analysis 56-45-1, L-Serine, analysis 56-84-8, L-Aspartic acid, analysis 56-85-9, L-Glutamine, analysis 56-86-0, L-Glutamic acid, analysis 56-87-1, L-Lysine, analysis 58-82-2, Bradykinin 60-18-4, L-Tyrosine, analysis 61-90-5, L-Leucine, analysis 63-68-3, L-Methionine, analysis 63-91-2, L-Phenylalanine, analysis **70-47-3**, Asn, analysis 71-00-1, L-Histidine, analysis 71-00-1D, Histidine, peptides contg. 72-18-4, L-Valine, analysis 72-19-5, L-Threonine, analysis 73-22-3, L-Tryptophan, analysis 73-22-3D, Tryptophan, peptides contg. 73-32-5, L-Isoleucine, analysis 74-79-3, L-Arginine, analysis 107-95-9, .beta.-Alanine 147-85-3, L-Proline, analysis 305-84-0, L-Carnosine 306-14-9 484-42-4 550-19-6 584-85-0, L-Anserine 1050-28-8 2390-74-1 2507-24-6, Physalemin 2577-40-4 2578-81-6 2990-43-4 3788-44-1 7390-78-5 7451-76-5 7763-65-7 9034-40-6, Luteinizing hormone-releasing hormone 9063-57-4, Tuftsin 9083-38-9, MSH-release inhibiting factor 11128-99-7, Angiotensin II 13602-53-4, Angiotensin III (human) 15266-88-3 15958-92-6 16870-37-4 16874-81-0 18048-87-8 19653-76-0 20696-60-0, Trp-Trp 20930-58-9 22677-56-1 22828-05-3 23403-90-9 24305-27-9, Thyrotropin-releasing hormone 25615-38-7 32999-80-7 33289-76-8 33507-63-0, Substance P 35979-00-1 38916-34-6, Somatostatin (sheep) 49557-75-7 55508-42-4, Neurotensin (ox) 55614-10-3, [Tyr8]-substance P 58822-25-6 59880-97-6 60815-41-0 62130-80-7 69431-45-4, Delta sleep-inducing peptide 71835-78-4 74032-87-4 111543-77-2 127441-82-1 131303-26-9 131374-17-9
RL: ANT (Analyte); ANST (Analytical study)
(**chromatog.** of, immobilized metal ion **affinity**)

IT 142-73-4
RL: ANST (Analytical study)
(immobilized metal ion **affinity chromatog.** in relation to)

IT 7647-14-5, Sodium chloride (NaCl), uses and miscellaneous
RL: USES (Uses)
(immobilized metal ion **affinity chromatog.** of peptides response to)

IT 7440-50-8D, Copper, conjugates with Chelating Superose or TSK gel chelate-5PW 101707-35-1D, TSK gel chelate-5PW, copper ion conjugates 131159-00-7D, Chelating Superose, copper ion conjugates
RL: ANST (Analytical study)
(peptides **sepn.** by **affinity chromatog.** on)

L18 ANSWER 22 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:41049 HCAPLUS
DOCUMENT NUMBER: 112:41049
TITLE: Controlled porous glass (CPG) with reactive epoxy groups as support for **affinity chromatography**. I. Optimization of CPG modification and the binding of glucose with modified surface
AUTHOR(S): Rogalski, J.; Dawidowicz, A. L.
CORPORATE SOURCE: Dep. Biochem., M. Curie-Sklodowska Univ., Lublin, 20-031, Pol.

SOURCE: Acta Biotechnologica (1989), 9(3), 275-83
CODEN: ACBTDD; ISSN: 0138-4988
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Controlled porosity Vycor glass (CPG) substrates were activated by treatment with .gamma.-aminopropyltriethoxysilane. The bifunctional oxirane diglycidoxy-diethyleneglycol ether was then coupled to the treated glass surface. Compds. having free hydroxyl and amine groups (e.g., saccharides and amino acids) can be coupled to the support: it is necessary to optimize the binding reaction for each compd. Hydroxyl-group compds. are more easily bonded than amino-group substances.

CC 57-1 (Ceramics)
Section cross-reference(s): 9, 33, 34

ST porous glass support **affinity chromatog**; glucose binding porous glass surface; silane activation porous glass surface; oxirane coupling porous glass surface

IT Glass, oxide
RL: USES (Uses)
(Vycor, porous supports from, modification of, for **affinity chromatog**.)

IT 919-30-2, .gamma.-Aminopropyltriethoxysilane 4206-61-5
RL: USES (Uses)
(activator, porous glass support modification by, for **affinity chromatog**.)

IT 56-41-7, Alanine, uses and miscellaneous 56-45-1, Serine, uses and miscellaneous 57-48-7, Fructose, uses and miscellaneous 59-23-4, Galactose, uses and miscellaneous 60-18-4, Tyrosine, uses and miscellaneous 63-42-3, Lactose 63-91-2, Phenylalanine, uses and miscellaneous 70-47-3, **Asparagine**, uses and miscellaneous
RL: USES (Uses)
(binding of, to porous glass supports, surface **modification** for improved, for **affinity chromatog**.)

L18 ANSWER 23 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1985:200536 HCAPLUS
DOCUMENT NUMBER: 102:200536
TITLE: Iron(3+)-hydroxamate as immobilized metal **affinity**-adsorbent for **protein chromatography**
AUTHOR(S): Ramadan, Nabil; Porath, Jerker
CORPORATE SOURCE: Inst. Biochem., Uppsala Biomed. Cent., Uppsala, S-751 23, Swed.
SOURCE: Journal of Chromatography (1985), 321(1), 93-104
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The adsorbent glycinehydroxamate-Sepharose 6B, charged with Fe3+ under specified conditions, is reported. It was used at various pH values for chromatog. of the following proteins: lysozyme, cytochrome c, avidin, bovine pancreatic RNase, myoglobin, ovalbumin and human serum albumin. The common naturally occurring amino acids were tested for their interactions with the new sorbent under neutral conditions.

CC 9-3 (Biochemical Methods)

ST iron hydroxamate **affinity chromatog protein**

IT **Proteins**
RL: ANT (Analyte); ANST (Analytical study)
(**chromatog**. of, **affinity**, iron-hydroxamate as stationary phase in)

IT Albumins, blood serum
Avidins
Myoglobins
Ovalbumins
RL: ANT (Analyte); ANST (Analytical study)
(**chromatog.** of, liq. **affinity,** iron-hydroxamate as stationary phase in)

IT **Chromatography,** column and liquid
(**affinity,** stationary phases, iron-hydroxamate as, for **protein sepn.**)

IT 52-90-4, analysis 56-40-6, analysis 56-41-7, analysis 56-45-1, analysis 56-84-8, analysis 56-85-9, analysis 56-86-0, analysis 56-87-1, analysis 56-89-3, analysis 60-18-4, analysis 61-90-5, analysis 63-68-3, analysis 63-91-2, analysis **70-47-3,** analysis 71-00-1, analysis 72-18-4, analysis 72-19-5, analysis 73-22-3, analysis 73-32-5, analysis 74-79-3, analysis 9001-63-2 9001-99-4 9007-43-6, analysis
RL: ANT (Analyte); ANST (Analytical study)
(**chromatog.** of, liq. **affinity,** iron-hydroxamate as stationary phase in)

L18 ANSWER 24 OF 24 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1982:48145 HCAPLUS
DOCUMENT NUMBER: 96:48145
TITLE: Kunitz-type proteinase inhibitors derived by limited proteolysis of the inter-.alpha.-trypsin inhibitor. V. Attachments of carbohydrates in the human urinary trypsin inhibitor isolated by **affinity chromatography**
AUTHOR(S): Hochstrasser, Karl; Schoenberger, Oeyvind L.; Rossmanith, Ingrid; Wachter, Elmar
CORPORATE SOURCE: Biochem. Labor Klin., Univ. Muenchen, Munich, Fed. Rep. Ger.
SOURCE: Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie (1981), 362(10), 1357-62
CODEN: HSZPAZ; ISSN: 0018-4888
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Trypsin inhibitor HI-30 of human urine, physiol. released from inter-.alpha.-trypsin inhibitor and having a known peptide sequence, was purified by affinity chromatog. and its carbohydrate structure was detd. The carbohydrates, which comprise .apprx.50 of the inhibitor, are attached to the peptide moiety at 2 sites. One chain is linked O-glycosidically via serine-10 in the N-terminal extension peptide and the other is linked N-glucosidically via arginine-24 in the inactive inhibitory Kunitz-type domain of the inhibitor. The complete sequences of the carbohydrate chains were detd.

CC 7-3 (Enzymes)

ST **affinity chromatog** trypsin inhibitor; trypsin inhibitor HI30 sequence urine; peptide sequence trypsin inhibitor HI30; carbohydrate sequence trypsin inhibitor HI30

IT **Protein** sequences
(of trypsin inhibitor HI-30, of urine)

IT 56-45-1, biological studies **70-47-3,** biological studies
RL: BIOL (Biological study)
(of trypsin inhibitor HI-30, carbohydrates linkage with)

```
=> fil wpids
FILE 'WPIDS' ENTERED AT 12:22:00 ON 24 APR 2003
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FILE LAST UPDATED:          07 APR 2003      <20030407/UP>
MOST RECENT DERWENT UPDATE:     200323        <200323/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

    Due to data production problems the WPI file had
    to be reset to update 200323.
    SDIs for update 24 will be rerun free of charge once
    the corrected data is loaded.
    Also answer sets created after April 10 may at least
    temporarily be affected and hence partially invalid.

>>> NEW WEEKLY SDI FREQUENCY AVAILABLE --> see NEWS  <<<

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    http://www.derwent.com/userguides/dwpi_guide.html <<<

=> d his

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               DEL HIS

     FILE 'STNGUIDE' ENTERED AT 12:12:47 ON 24 APR 2003

     FILE 'WPIDS' ENTERED AT 12:13:03 ON 24 APR 2003
               E WO2000023580
               E WO2000023580/PN

     FILE 'STNGUIDE' ENTERED AT 12:14:23 ON 24 APR 2003

     FILE 'WPIDS' ENTERED AT 12:16:36 ON 24 APR 2003
L2          4577 S AFFIN?  (S) (SEPN# OR SEPARAT? OR CHROMAT?)
L3        124391 S PROTEIN# OR LIGAND#
L4          5429 S ASN OR ASPARAGINE#
L5            66 S L2 AND L3 AND L4
L6            48 S L5 AND D16/DC
L7           760 S L4 (L) (ALTER? OR MODIF?)
L8            14 S L6 AND L7
L9          7513 S (ALK OR ALKALINE?) (S) (RESIST? OR SENSITIV?)
L10            2 S L6 AND L9
L11       104089 S ALK OR ALKALINE#
L12            4 S L6 AND L11
L13           16 S L8 OR L10 OR L12
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FILE 'WPIDS' ENTERED AT 12:22:00 ON 24 APR 2003

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                 OR CHROMAT?)
L3        124391 SEA FILE=WPIDS ABB=ON  PLU=ON  PROTEIN# OR LIGAND#
L4          5429 SEA FILE=WPIDS ABB=ON  PLU=ON  ASN OR ASPARAGINE#
L5            66 SEA FILE=WPIDS ABB=ON  PLU=ON  L2 AND L3 AND L4
L6            48 SEA FILE=WPIDS ABB=ON  PLU=ON  L5 AND D16/DC
L7           760 SEA FILE=WPIDS ABB=ON  PLU=ON  L4 (L) (ALTER? OR MODIF?)
L8            14 SEA FILE=WPIDS ABB=ON  PLU=ON  L6 AND L7
L9          7513 SEA FILE=WPIDS ABB=ON  PLU=ON  (ALK OR ALKALINE?) (S) (RESIST?
                 OR SENSITIV?)
L10            2 SEA FILE=WPIDS ABB=ON  PLU=ON  L6 AND L9
L11       104089 SEA FILE=WPIDS ABB=ON  PLU=ON  ALK OR ALKALINE#
L12            4 SEA FILE=WPIDS ABB=ON  PLU=ON  L6 AND L11
L13           16 SEA FILE=WPIDS ABB=ON  PLU=ON  L8 OR L10 OR L12

=> d .wp 1-16 l13

L13  ANSWER 1 OF 16  WPIDS (C) 2003 THOMSON DERWENT
AN   2003-210180 [20]   WPIDS
DNC  C2003-053560
TI   New protein for preventing or treating vascular and endothelial
     diseases, e.g. cancer, myocardial infarction, thrombotic diseases,
     inflammation, ischemia, retinopathies, asthma, wounds, diabetes or stroke.
DC   B04 D16 P14
IN   FARADAY, N; HIEMISCH, H; KRUPP, E; LANAHAN, A; REGARD, J B; SCHEEK, S;
     SCHWANINGER, M; WORLEY, P F
PA   (AXAR-N) AXARON BIOSCIENCE AG
CYC  100
PI   WO 2003001863 A2 20030109 (200320)* EN  114p
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM
            ZW
     DE 10130657   A1 20030116 (200320)
ADT  WO 2003001863 A2 WO 2002-EP6770 20020619; DE 10130657 A1 DE 2001-10130657
     20010627
PRAI DE 2001-10130657 20010627
AB   WO2003001863 A UPAB: 20030324
     NOVELTY - A protein comprising:
          (a) any of the 4 fully defined sequences of 246 (S1-3) or 297 (S4)
     amino acids given in the specification;
          (b) a sequence which can be obtained by substitution, insertion or
     deletion of one or more amino acid residue(s) of S1-4, with at least one
     of the essential properties of S1-4 being retained; or
          (c) a functional equivalent or functionally equivalent part of S1-4.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
          (1) a nucleic acid sequence encoding the above protein;
          (2) a nucleic acid construct comprising the above nucleic acid
     sequence linked to at least one genetic regulatory element;
          (3) a transgenic, non-human organism transformed with the functional
     or non-functional transgenic nucleic acid sequence or construct cited
```

above;

(4) processes for finding compounds having specific binding affinity for the above **protein** or nucleic acid, or compounds which modulate or normalize at least one essential property, or the expression, of the above **protein**;

(5) a compound obtained by method (4); and

(6) processes for qualitatively or quantitatively detecting the presence, absence, incorrectly regulated expression or an incorrect function of the above **protein** or nucleic acid sequence in a biological sample.

ACTIVITY - Vasotropic; Anticoagulant; Thrombolytic; Anticonvulsant; Antiarthritic; Antipsoriatic; Cytostatic; Antiinflammatory; Antiasthmatic; Ophthalmological; Cardiant; Cerebroprotective; Nephrotropic; Vulnerary; Antidiabetic.

No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The **protein** and the nucleic acid sequence or construct are useful in identifying **proteins** which possess specific binding affinities for the **protein** cited above, for identifying nucleic acids which encode **proteins** possessing specific binding affinities for the **protein** cited above, and for producing drugs (claimed). The nucleic acid sequence or its fragment may also be used for isolating a genomic sequence by means of screening for homology, as a marker for human hereditary diseases, or for detecting sequence polymorphisms that correlate with predispositions to diseases (claimed).

The **protein**, nucleic acid sequence or construct, and the transgenic animal may also be used for the treatment of human diseases by gene therapy (claimed). These may be used in preventing or treating vascular, endothelial, coagulation, thrombotic and/or platelet diseases, such as epilepsy, cancer, inflammation, ischemia, retinopathies, asthma, acute myocardial infarction, stroke, arthritis, psoriasis, kidney diseases, wounds, diabetes and lung diseases.

Dwg.0/40

TECH UPTX: 20030324

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred **Protein**: The **protein** comprises the sequence motifs (I)-(VIII):

(I) Asp-Ala-Leu-Arg-Arg-Phe-Gln-Gly-Leu-Leu-Leu-Asp-Arg-Arg-Gly-Arg-Leu-His-;

(II) Gln-Val-Leu-Arg-Leu-Arg-Glu-Val-Ala-Arg-Arg-Leu-Glu-Arg-Leu-Arg-Arg-Arg-Ser-Leu-;

(III) Gly-Ala-Leu-Ala-Ala-Ile-Val-Gly-Leu-Ser-Leu-Ser-Pro-Val-Thr-Leu-Gly-;

(IV) Ser-Ala-Val-Gly-Leu-Gly-Val-Ala-Thr-Ala-Gly-Gly-Ala-Val-Thr-Ile-Thr-Ser-Asp-Leu-Ser-Leu-Ile-Phe-Cys-**Asn**-Ser-Arg-Glu-;

(V) Arg-Arg-Val-Gln-Glu-Ile-Ala-Ala-Thr-Cys-Gln-Asp-Gln-Met-Arg-Glu-;

(VI) Ala-Leu-Tyr-**Asn**-Ser-Val-Tyr-Phe-Ile-Val-Phe-Phe-Gly-Ser-Arg-Gly-Phe-Leu-Ile-Pro-Arg-Arg-Ala-Glu-Gly-;

(VII) Thr-Lys-Val-Ser-Gln-Ala-Val-Leu-Lys-Ala-Lys-Ile-Gln-Lys-Leu-; or

(VIII) Glu-Ser-Leu-Glu-Ser-Cys-Thr-Gly-Ala-Leu-Glu-Leu-Ser-Glu-Gln-Leu-Glu-Ser-Arg-Val-Gln-Leu-Cys-Thr-Lys-.

Preferred Nucleic Acid: The nucleic acid comprises any of the 6 fully defined sequences of 741-16613 bp (S5-10) given in the specification, or a sequence that is at least 60% identical to S5-10. It also encodes a **protein** which has at least 60% identity with S1-4.

Preferred Transgenic Organism: The organism is an animal in whose germ cells, or the entirety or a part of the somatic cells, the nucleic acid sequence cited above has been transgenically **altered** by

recombinant methods or interrupted by inserting DNA elements. Preferred Process: Finding compounds having specific binding **affinity** for the above **protein** or nucleic acid, comprises incubating the **protein** or nucleic acid with the compound to be tested, and detecting the binding of the compound to the **protein** or nucleic acid. Finding compounds which modulate or normalize at least one essential property, or the expression, of the above **protein**, comprises incubating the **protein**, nucleic acid sequence or construct, or the transgenic animal, with the compound to be tested, and determining the modulation or normalization of an essential property, or of the expression, of the **protein** cited above. The modulation or normalization of an essential property is determined by direct binding of the compound to be tested to the **protein**, nucleic acid sequence or construct. The method employs an immunoprecipitation, N-hybrid system, phage display system, a library of low molecular weight compounds, a reporter system, antibody selection techniques, immunoassays (e.g. ELISA or Western blotting), molecular modeling using the structural information for the **protein** or nucleic acid sequence, **affinity chromatography**, or microphysiometer.
Preferred Compound: The compound is a **protein**, nucleic acid, or low molecular weight compound having a molecular weight of less than 1000 g/mol. In particular, the compound is selected from polyclonal or monoclonal antibodies, antibody mixtures, single-chain antibodies or antibody fragments, aptamers, natural or artificial transcription factors, antisense nucleic acids, double-stranded RNA molecules, alpha-anomeric nucleic acids, low molecular weight compounds and ribozymes. Qualitatively or quantitatively detecting the presence, absence, incorrectly regulated expression or an incorrect function of the above **protein** or nucleic acid sequence in a biological sample, comprises isolating a biological sample from a test subject, and incubating the sample with a reagent suitable for detecting the above **protein** or nucleic acid sequence, in a manner such that the presence, absence, or the incorrectly regulated expression or an incorrect function of the above **protein** or nucleic acid sequence can be detected. Qualitatively or quantitatively detecting the nucleic acid cited above in a biological sample, comprises incubating a biological sample with a known quantity of the nucleic acid or oligonucleotides which can be used as primers for amplifying the nucleic acid or mixtures cited above, detecting the nucleic acid by specific hybridization or polymerase chain reaction (PCR) amplification, and comparing the quantity of hybridizing nucleic acid or of the nucleic acid obtained by PCR amplification with a standard. Qualitatively or quantitatively detecting the **protein** cited above in a biological sample, comprises incubating a biological sample with an antibody that is specifically directed against the **protein**, detecting the antigen/antibody complex, and comparing the quantities of the complex with a quantity standard.

L13 ANSWER 2 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2003-201298 [19] WPIDS
DNC C2003-051140
TI Peptide analog useful for e.g. studying beta-sheet nucleation comprises a peptide in which at least one amino acid, but less than all amino acids is replaced by an azacyclohexenone group.
DC B03 B04 **D16**
IN BARTLETT, P A; OLSON, S; PHILLIPS, S; REZAC, M
PA (REGC) UNIV CALIFORNIA
CYC 99

PI WO 2002099045 A2 20021212 (200319)* EN 31p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
ADT WO 2002099045 A2 WO 2002-US17401 20020530
PRAI US 2001-296167P 20010605
AB WO 200299045 A UPAB: 20030320
NOVELTY - A peptide analog (a) comprises a peptide in which at least one amino acid, but less than all amino acids is replaced by an azacyclohexenone (Ach) group.

DETAILED DESCRIPTION - A peptide analog comprises a peptide in which at least one amino acid, but less than all amino acids is replaced by an azacyclohexenone (Ach) group of formula (i).

INDEPENDENT CLAIMS are also included for:

(1) Compounds of formulae (I)-(V), amine-protected analogs of (II)-(V) that terminate with H2N-, carboxy-protected analogs of (II) - (V) that terminate with -CO2H, carboxy-activated analogs of (II) - (V) that terminate with -CO2H, amine-protected and carboxy-protected analogs of (V), and amine-protected and carboxy-activated analogs of (V);

(2) A peptide analog (a1) of formula (VI);

(3) A peptide analog (a2) comprising a first segment and second segment consisting of a sequence of amino acids joined by amide bonds and in the second segment at least one amino acid, but less than all amino acids are replaced by (i). The first and second segments are joined by a covalent linkage that permits the first and second segments to adopt a beta -sheet like interaction;

(4) Extraction of a target peptide having a selected amino acid sequence from a mixture of peptides involving contacting the mixture with a capture peptide that is covalently bonded to the solid support and associates with the amino acid sequence in a beta -sheet interaction. The capture peptide comprises amino acid and at least one (i).

R1 = protecting group other than methyl or ethyl;
R2 = OH or activated leaving group;
R11, R12 and R21 = amino acid side chain;
R13 = H or amine protecting group;
R14 = H or carboxy protecting group;
R22 = peptide chain terminating group or group of formula (ii);
R24 = H, alkyl, acyl, carbamoyl or alkoxycarbonyl;
R23 = peptide chain terminating group or group of formula (iii);
R25 = OH, alkoxy, alkylamino, dialkylamino or arylamino;
n = at least 2.
asterisk denotes the site of attachment.

ACTIVITY - Nootropic; Neuroprotective; Antiallergic; Antipsoriatic; Antiarthritic; Antiarteriosclerotic; Cytostatic; Virucide.

MECHANISM OF ACTION - Inflammatory cytokine blocker.

USE - For inhibiting the association of a selected peptide with the other peptides; for inhibiting the association of a peptide with a double-stranded nucleic acid; for inhibiting the biological activity of a peptide; for increasing the tendency of a target peptide or a portion of a target peptide to assume a beta -strand conformation; for extracting a target peptide having a selected amino acid sequence from a mixture of peptide (all claimed); for studying beta -sheet nucleation, propagation and suppression; as prophylactic or palliative agents in physiological conditions that involve or are controllable by beta -sheet interactions; in the treatment of prion disease or Alzheimer's disease; for blocking the

infectivity of the human immunodeficiency virus by inhibiting the association of the viral gp 120 **protein** with the CD4 receptor on the T-lymphocyte cell surface; for treating allergic reactions, psoriasis, arthritis, atherosclerosis, multiple sclerosis and cancer; as **protein** purification media in **affinity chromatography**; as component in diagnostic devices or kits; for the concentration and identification of peptide and **protein** analytes; for therapeutic effects by complexing with the blocking the action of specific peptide hormones or by targeting attached radiopharmaceuticals or cytotoxic agents to the specific sites in the body; in an array such as that of a proteomics chip for use in an assay for the levels of expression of specific **proteins** in different tissues and under different conditions.

ADVANTAGE - A beta -sheet-like interaction is achieved between the peptide and peptide analog. The peptide analogs as well the Ach groups are simpler to synthesize than the peptide mimics of the prior art. The side-chain interactions between the peptide analog and the peptide provide sequence selectivity. The Azh-containing portion of the construct has a strong tendency to enter into a stable beta -sheet-like interaction thus stabilizing the conventional peptide portion in the beta -strand conformation that serves as a template for beta -sheet-like interactions with other peptides. The analogs have enhanced ability to an exposed surface of the amyloid beta -sheet complex and prevent further aggregation.

Dwg.0/5

TECH UPTX: 20030320

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - Preferred Compound: The compound is a compound of formula (II) or its amine-protecting analog, a compound of formula (III) or its carboxy-protected or carboxy-activated analog, compound of formula (V) or its amine-protected, carboxy-protected, carboxy-activated, amine-protected and carboxy-protected, or amine-protected and carboxy-activated analog.

Preferred Analog: (a) has amino acids (2-200, preferably 2-100, more preferably 2-20, especially 2-10) and Ach group (1-100, preferably 1-50, especially 1-20) in number. The ratio of Ach to amino acids is 1:10-10:1 (preferably 1:5-5:1, especially 1:2-1:1). The second segment consists of an amino acid sequence, in which at least two non-adjacent amino acids are replaced by Ach groups. In at least a portion of the second segment, Ach group replaces every second amino acid. The first segment contains amino acids (3-200, preferably 3-100, especially 3-20) and in the second segment total number of amino acids and Ach groups is 3-200 (preferably 3-100, especially 3-20 or 4-10). The covalent linkage is D-Pro-Ala or **Asn** -Gly. The capture peptide comprises amino acids and at least two non-adjacent Ach group. In at least a portion of the capture peptide comprises amino acid **alternating** with Ach group (at least 2).

The total number of amino acids and Ach groups in the capture peptide is 3-200 (preferably 4-20).

L13 ANSWER 3 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2003-167361 [16] WPIDS
DNN N2003-132257 DNC C2003-043491
TI Assaying a woman for ovarian cancer, useful as a screening tool or in helping to confirm a diagnosis or for monitoring the disease and the effectiveness of therapy, comprises determining the levels of the human haptoglobin alpha subunit.
DC B04 **D16** P31
IN BERKOWITZ, R S; CRAMER, D W; MOK, S C; SKATES, S; YE, B
PA (BGHM) BRIGHAM & WOMENS HOSPITAL INC

CYC 23
PI WO 2002100242 A2 20021219 (200316)* EN 21p
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: AU CA JP
US 2003017515 A1 20030123 (200316)
ADT WO 2002100242 A2 WO 2002-US17845 20020606; US 2003017515 A1 Provisional US 2001-296458P 20010608, US 2002-162221 20020605
PRAI US 2001-296458P 20010608; US 2002-162221 20020605
AB WO2002100242 A UPAB: 20030307
NOVELTY - Assaying a woman for ovarian cancer, comprising determining the levels of the human haptoglobin alpha subunit, is new.

DETAILED DESCRIPTION - Assaying a woman for ovarian cancer, comprising:

(a) obtaining a test biological sample from the woman or patient;

(b) assaying the test biological sample to determine the amount of human haptoglobin alpha subunit or human haptoglobin alpha antigen that is present;

(c) comparing the results in step (B) with the amount in a control sample or with the results of assays previously performed for the woman; and

(d) assessing the degree to which the ovarian cancer is likely present based upon the amount of haptoglobin alpha subunit in the biological test sample relative to the level in the control sample or in the assays previously performed for the woman.

Alternatively, the method comprises:

(a) obtaining a test biological sample from the woman;

(b) performing surface enhanced laser desorption/ionization mass spectroscopy on the test biological sample in which the test biological sample is adsorbed on an immobilized metal affinity capture **protein** chip;

(c) determining the extent to which the mass spectroscopy produces a spectrum with a peak of 11600-11700 (m/z); and

(d) assessing the degree to which the ovarian cancer is likely present based upon the presence and size of the peak.

INDEPENDENT CLAIMS are included for the following:

(1) an antibody made by a process comprising:

(a) injecting an animal with a peptide having the sequence of (S1); or

(b) obtaining a serum or plasma sample from a patient with ovarian cancer, isolating a **protein** that produces a peak of 11600-11700 (m/z) by mass spectroscopy and which has an amino acid sequence corresponding to a sequence of the alpha subunit of human haptoglobin, and injecting the isolated peptide into an animal capable of making the antibody;

(2) an enzyme linked immunosorbent assay (ELISA) kit for assaying a sample for human haptoglobin alpha antigen, comprising:

(a) a first antibody that binds to intact human haptoglobin; and

(b) a second antibody that binds with specificity to an epitope on the human haptoglobin alpha chain but which does not bind to intact human haptoglobin that has been bound by the first antibody; and

(3) assaying a test sample for human haptoglobin alpha antigen by ELISA comprising:

(a) immobilizing the human haptoglobin alpha antigen by incubating the test sample on a microtiter plate;

(b) incubating the immobilized antigen with a first antibody that binds to intact human haptoglobin;

(c) incubating the preparation of step (b) with a second antibody that binds with specificity to an epitope on the human haptoglobin alpha

chain but which does not bind to intact human haptoglobin that has been bound by the first antibody;

(d) incubating the preparation of step (c) with a third antibody that is conjugated to an enzyme that can be used for quantifying binding, where the third antibody recognizes and binds the second antibody but not the first antibody; and

(e) quantifying the amount of haptoglobin alpha antigen present by carrying out a reaction catalyzed by the enzyme conjugated to the third antibody.

Asn-Asn-Lys Lys-Gln-Trp-Ile-**Asn**-Lys Ala-Val-Gly Asp-Lys-Leu-Pro-Glu-Cys (S1).

USE - The method is useful for determining whether a woman has, or is likely to develop, ovarian cancer. Assaying a woman for ovarian cancer is useful as a screening tool or in helping to confirm a diagnosis, or for monitoring the disease and the effectiveness of therapy.

Dwg.0/0

TECH UPTX: 20030307

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Assaying a woman for ovarian cancer, where the test biological samples and control samples are serum, plasma or whole blood. The sample is assayed for haptoglobin alpha antigen using an immunoassay, which is a radioimmunoassay or an ELISA. A divalent cationic metal activates the immobilized metal **affinity** capture chip, where the cationic metal is Cu2+. Assaying a test sample for human haptoglobin alpha antigen by ELISA, where the first antibody is monoclonal, and the second antibody is polyclonal. The test sample is human serum, plasma or blood. The enzyme is horseradish peroxidase.

Preferred Process: The process of making an antibody further comprises isolating the antibody from the serum or plasma of the animal.

Preferred Kit: The first antibody is monoclonal, and the second antibody is polyclonal. The second antibody is made by the process of injecting an animal with the peptide having the sequence of S1. The kit further comprises:

(a) Intact human haptoglobin or human haptoglobin beta chain in a container **separate** from the antibody or human haptoglobin alpha subunit; and

(b) Human haptoglobin alpha subunit or a peptide having at least 10 consecutive amino acids corresponding to a sequence found within that of the human haptoglobin alpha chain, in a container **separate** from the antibody and the intact human haptoglobin or human haptoglobin beta chain.

L13 ANSWER 4 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2003-031166 [03] WPIDS
DNC C2003-007275
TI New isolated peptide, useful as affinity purification tag for recombinant **protein**, comprises at least two high-affinity streptavidin-binding modules.
DC B04 **D16**
IN SCHMIDT, T
PA (BIOA-N) INST BIOANALYTIK GMBH GOETTINGEN; (IBAI-N) IBA GMBH
CYC 97
PI DE 10113776 A1 20021002 (200303)* 17p
WO 2002077018 A1 20021003 (200303) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO

RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

ADT DE 10113776 A1 DE 2001-10113776 20010321; WO 2002077018 A1 WO 2001-EP11846 20011012

PRAI DE 2001-10113776 20010321

AB DE 10113776 A UPAB: 20030113

NOVELTY - Isolated peptide (I) comprising at least two individual modules (M), separated by 0-50 amino acids (aa), with each containing at least one motif His-Pro-X where X = Gln, **Asn** or Met, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) fusion **protein** (FP) comprising (I) attached to a **protein** (II);

(2) expression vector containing a nucleic acid (III) that encodes (I) and includes, 5' and/or 3' to (III), a restriction site for insertion of a sequence that encodes (II), or its fragment, to be expressed;

(3) preparation (M1) of a recombinant FP by inserting the sequence for FP into a host cell, cell lysate or cell extract;

(4) detection (M2) and/or recovery of FP by contacting a sample with a conjugate of streptavidin (S) with a label and/or carrier;

(5) nucleic acid encoding (III); and

(6) use of (S) and/or its muteins as receptor for binding (I).

USE - (I), which contain streptavidin (S) binding modules, are useful as affinity handles for purification of recombinant fusion **proteins** (FP), also for detecting FP, e.g. on **protein** chips or microtiter plates.

ADVANTAGE - The modules in (I) bind strongly to streptavidin, with a co-operative effect that provides stronger binding than a single tag, but are displaced by a competitor. (I) does not interfere with the function of attached **proteins** (II) (so it may not be essential to remove it); facilitates detection, and has easily controllable binding properties. (I) is particularly used for purifying fusion **proteins** (FP) from dilute solution in batch formats (which use simpler apparatus than column methods and result in lower loss of FP).

Dwg.0/1

TECH UPTX: 20030113

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Process: (M1) involves incorporating a vector that contains the sequence for FP into a host cell. In (M2), the label is a fluorophore and/or enzyme, specifically **alkaline** phosphatase or horseradish peroxidase, and isolation of (II) bound to (I) comprises subjecting the sample to S-**affinity** **chromatography** to form a complex between S, or its mutein, and (I). The bound **protein** is then eluted with a **ligand** for S or its mutein.

TECHNOLOGY FOCUS - BIOLOGY - Preferred Peptide: At least one M includes the sequence His-Pro-Gln, especially the sequence O-X'-His-Pro-Gln-Phe-Y-Z.

O = Trp, Lys or Arg;

X' = any aa;

both Y and Z = Gly, or Y = Glu and Z = Lys or Arg.

Most preferably (I) contains the sequence Trp-Ser-His-pro-Gln-Phe-Glu-Lys-(X')n-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys where n = 8-12 and X'n = 2 or 3 repeats of GlyGlyGlySer.

Preferred **Protein**: In FP, (II) is a complete **protein**; a mutant (preferably deletion or substitution mutant), or a **protein** fragment, and FP may include, between (I) and (II), a linker containing a proteolytic cleavage site.

Preferred Materials: The **ligand** for S is Trp-X'-His-Pro-Gln-Phe-Y-Z or any of 2-iminobiotin; lipoic acid; (dimethyl)hydroxyphenylazobenzoi

c acid; diaminobiotin and/or dethiobiotin.

L13 ANSWER 5 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-608378 [65] WPIDS
CR 2001-607518 [69]; 2002-010593 [01]; 2002-010610 [01]
DNC C2002-171980
TI Identifying a binding compound for cysteine-X-cysteine chemokine receptor 4 (CXCR4) for preventing or treating AIDS and HIV infection, comprises binding a molecule to a molecule with a binding property corresponding to CXCR4.
DC B04 **D16**
IN KATES, S A; KRSTENANSKY, J; NESTOR, J J; TAN HEHIR, C A; WILSON, C J
PA (CONS-N) CONSENSUS PHARM INC
CYC 22
PI WO 2002057313 A2 20020725 (200265)* EN 92p
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: AU CA JP
ADT WO 2002057313 A2 WO 2001-US51165 20011026
PRAI US 2001-813653 20010320; US 2000-243587P 20001027; US 2001-813448 20010320; US 2001-813651 20010320
AB WO 200257313 A UPAB: 20021010
NOVELTY - Identifying (M1) a binding compound for cysteine-X-cysteine chemokine receptor 4 (CXCR4) comprises binding a molecule from the library of two or more molecules to the molecule with a binding property corresponding to CXCR4.

DETAILED DESCRIPTION - Identifying (M1) a binding compound for cysteine-X-cysteine chemokine receptor 4 (CXCR4) comprises binding a molecule from the library of two or more molecules to the molecule with a binding property corresponding to CXCR4. (M1) comprises:

(a) providing a library of two or more molecules;

(b) providing a molecule with a binding property corresponding to CXCR4;

(c) binding a molecule from the library of two or more molecules to the molecule with a binding property corresponding to CXCR4;

(d) separating the bound molecule from the library of two or more molecules; and

(e) identifying the bound molecule as a binding compound for CXCR4.

INDEPENDENT CLAIMS are also included for the following:

(1) a binding compound (I) for CXCR4 identified from M1;

(2) preventing (M2) HIV infection or AIDS in a patient by administering a composition comprising (I) in a physiological carrier;

(3) treating (M3) or preventing AIDS in a patient by administering a therapeutic composition comprising (I) in a controlled release injectable formulation;

(4) a computer-aided method (M4) for identifying relative binding affinity of a test molecule to CXCR4;

(5) determining (M5) an amino acid sequence motif for an interaction site of a binding compound for CXCR4;

(6) amino acid sequence motifs (II) for a binding compound for CXCR4 identified from (M5);

(7) binding compounds (III) identified from (M1), and binding compounds with the amino acid sequence motif for CXC chemokine receptor as determined by (M5);

(8) a library (IV) comprising members based upon an amino acid sequence motif for an interaction site of a binding compound for CXCR4, where the motif is determined by permitting at least one peptide member from a peptide library to interact with the binding compound for CXCR4, and determining an amino acid sequence of at least one peptide that

interacts with the binding compound for CXCR4;

(9) solubilizing (M6) or immobilizing a compound corresponding to the binding property of CXCR4;

(10) a CXC chemokine 4 transfer vector (V) comprising a CXCR4 molecule and a tag selected consisting of GST, FLAG, 6 multiply His, C-MYC, MBP, V5, Xpress, CBP or HA;

(11) using (M7) a 3-dimensional structure of CXC chemokine receptor 4 in a drug screening assay; and

(12) treating (M8) a disease by administering a composition containing a compound of (M1) and a physiological carrier.

ACTIVITY - Virucide; Anti-HIV.

No suitable data given.

MECHANISM OF ACTION - Cysteine-X-Cysteine-Chemokine-Receptor-Binding-Antagonist-4; Cysteine-X-Cysteine-Chemokine-Receptor-Binding-Agonist-4.

USE - The partially purified CXCR4 **protein** is useful for carrying out selection, identification, and improvement of tight binding **ligands** in identifying therapeutically useful compounds. Therapeutic peptides, peptidomimetics, or small molecule antagonists or agonists of CXCR4 binding may be used in the prevention and treatment of AIDS and HIV infection.

Dwg.0/13

TECH UPTX: 20021010

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The library of two or more molecules is selected from linear peptides, cyclic peptides, natural amino acids, unnatural amino acids, peptidomimetic compounds and small molecule compounds. The molecule with a binding property corresponding to CXCR4 is a partially purified CXC chemokine receptor, or comprises a CXCR4 molecule and a tag selected from GST, FLAG, 6xHis, C-MYC, MBP, V5, Xpress, CBP, and HA.

The two or more molecules from the library are a peptide, a peptidomimetic or small molecule that can substituted for a **protein** capable of binding to receptors, enzymes or other **proteins**. These molecules comprise a molecule with an antagonistic effect on CXCR4 binding activity. The library comprises a phage library. (M1) further comprises solubilizing the molecule with a binding property corresponding to CXCR4 substantially in the absence of sodium chloride or using a buffer with a low salt concentration. Steps (a)-(d) are repeated at least once prior to the step (e).

(M4) comprises:

(a) entering input data characterizing CXCR4 into a computer program;

(b) entering input data characterizing at least one test peptide-like molecule, each of known sequence but unknown binding **affinity**; and analyzing each applied test peptide-like molecule using the computer program to generate a prediction of a relative binding **affinity** for each test peptide-like molecule, and outputting such prediction.

(M5) comprises:

(a) contacting a peptide library with a molecule with a binding property corresponding to CXCR4 under conditions which allow interaction between the molecule with a binding property corresponding to CXCR4 and the peptide library;

(b) allowing the molecule with a binding property corresponding to CXCR4 to interact with the peptide library such that a complex is formed between the molecule with a binding property corresponding to CXCR4 and a subpopulation of library members capable of interacting with the molecule with a binding property corresponding to CXCR4;

(c) **separating** the subpopulation of library members capable of interacting with the molecule with a binding property corresponding to CXCR4 from library members that are incapable of interacting with the

molecule with a binding property corresponding to CXCR4;
(d) determining a relative abundance of different amino acid residues at each degenerate position within the subpopulation of library members; and
(e) determining an amino acid sequence motif for an interaction site of the molecule with a binding property corresponding to CXCR4, based upon the relative abundance of different amino acid residues at each degenerate position within the library members.
Preferably, at least one member of the peptide library comprises at least one unnatural amino acid, where the peptide library comprises at least one molecule selected consisting of linear peptides, cyclic peptides, natural amino acids, unnatural amino acids, peptidomimetic compounds and small molecule compounds. The molecule with a binding property corresponding to CXCR4 group consists of linear peptides, cyclic peptides, natural amino acids, unnatural amino acids, peptidomimetic compounds and small molecule compounds. The peptide library is selected from a pre-determined CPI peptide sequence. The peptide library is selected from (S1-7).
(M6) is conducted substantially in the absence of sodium chloride when determining a compound corresponding to the binding of CXCR4.
Alternatively, the method is conducted using a low salt concentration when determining a compound corresponding to the binding of CXCR4. The salt concentration comprises a pre-determined amount of magnesium and calcium.
(M7) comprises:
(1) selecting a potential drug by performing rational drug design with the 3-dimensional structure, where selection is performed in conjunction with computer modeling;
(2) contacting the potential drug with a first molecule comprising a first CXCR4; and
(3) detecting the binding of the potential drug with the first molecule, where a potential drug is selected as a drug if the potential drug binds to the first molecule. The first molecule is labeled and is bound to a solid support.
Preferred Sequence Motifs: The amino acid sequence motif as determined by (M5) is (S8).
Preferred Binding Compounds: The binding compound identified in M1 has a sequence selected from (S9-S18). The binding compound may also have the sequence A'-B'-C'-D'-E'-E'-F'-C'-G'-F'/C'/B'-C'-B/C'-F'/C'-C'-C'. The binding compound may further comprise a sequence selected from (S19-S28).
Met-X-X-X-Arg-X-X-X-X-Ala (S1)
Met-Ala-X-X-X-X-Arg-X-X-X-X-Lys-Lys-Lys (S2)
Met-Ala-X-X-X-X-Trp-X-X-X-X-Ala-Lys-Lys-Lys (S3)
Met-Ala-Arg-X-X-Ile-Trp-Arg-X-X-X-Ala-Lys-Lys-Lys (S4)
Met-X-X-X-X-Trp-X-X-X-X-Ala-Lys-Lys-Lys (S5)
cyclo(Met-X-X-X-X-Arg-X-X-X-X-**Asn**) (S6)
cyclo(Met-Lys-X-Asp-His-Arg-X-X-Lys-**Asn**) (S7)
Met -Ala-Arg-Ser-Leu-Ile-Trp-Arg-Pro-Ala-Lys-Ala-Lys-Lys-Lys (S8)
Pro-Ala-His-Tyr-Pro-Met-Leu (S9)
Gln-Tyr-Ala-Thr-Pro-**Asn**-Lys (S10)
Gln-Gln-Arg-Ser-Thr-Ala-Phe (S11)
Pro-Phe-Arg-Ala-Thr-Thr-Glu (S12)
Thr-Asp-Lys-Leu-Leu-Leu-Asp (S13)
His-Thr-Gln-His-V-Arg-Thr (S14)
Leu-G-V-Lys-Ala-Pro-Ser (S15)
Asp-Leu-Gln-Ala-Arg-Tyr-Ser (S16)
Ser-Leu-Thr-Glu-Pro-Ser-Leu (S17)
Ser-Thr-Trp-Pro-Leu-Ala-Gln (S18)
Arg-Thr-Thr-Ser-Asp-Ala-Leu (S19)
Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-(Thric)-Ala-Arg-Arg-(2-Nal)-Arg-Arg-

Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-Pro-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S20)
Lys-Lys-Lys-Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-Leu-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S21)
Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-Ala-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S22)
Arg-Arg-Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-Ala-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S23)
His-Ala-Arg-Ser-Leu-Ile-(2-Nal)-Arg-His-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S24)
Cyclo (Glu0, Lys4)-Glu-Met-Ala-Arg-Lys-Leu-Ile-(2-Nal)-Arg-(Thric)-Ala-Arg-Arg-(2-Nal)-Arg-Arg (S25)
Cyclo (Glu8, Lys12)-Ala-Arg-Ser-Leu-Ile-(2-Nal)-Glu-(Thric)-Arg-Ala-Lys-(2-Nal)-Arg-Arg (S26)
Cyclo (Asp-Cys8, Cysys11)-Ala-Arg-Ser-Leu-Ile-(2-Nal)c(Thric)-Arg-Cys-Arg-(2-Nal)-Arg-Arg (S27)
Cyclo (Glu0, Lys4)-Glu-Met-Ala-Arg-Lys-Leu-Ile-Trp-Arg-Pro-Ala-Lys-Ala-Lys-Lys-Lys (S28)

L13 ANSWER 6 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-599760 [64] WPIDS
DNN N2002-475470 DNC C2002-169580
TI Novel trifunctional synthetic reagents for labeling peptides at specific amino acid residue and selectively enriching only those peptides containing labeled amino acid, useful for proteomic analysis.
DC B04 B05 **D16** S03
IN ANDON, N; HAYNES, P; WEI, J; YATES, J
PA (SYGN) SYNGENTA PARTICIPATIONS AG
CYC 96
PI WO 2002059144 A2 20020801 (200264)* EN 79p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU DM DZ EC ES GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
ADT WO 2002059144 A2 WO 2002-US2487 20020125
PRAI US 2001-305232P 20010713; US 2001-264576P 20010126
AB WO 200259144 A UPAB: 20021007
NOVELTY - Analytical reagents e.g. trifunctional synthetic reagents which can be used for reducing the complexity of peptide mixtures by labeling peptides at specific amino acid residue and then selectively enriching only those peptides containing the labeled amino acid, are new.
DETAILED DESCRIPTION - A compound (C1) of formula immobilization site-cleavage site-link (I) where:
(a) immobilization site is chosen from an epitope tag, a linker to a solid surface, a metal chelating site, a magnetic site, and a specific oligonucleotide sequence, or their combination;
(b) cleavage site is chosen from a protease cleavage site, a photocleavable linker, a restriction enzyme cleavage site, a chemical cleavage site, and a thermal cleavage site, or their combination; and
(c) link is chosen from an amino acid reactive site and a mass variance site, or their combination.
INDEPENDENT CLAIMS are also included for the following:
(1) a compound (C2) of formula II or III;
(2) preparing a fusion **protein** of formula (VI), by preparing a fusion **protein** sample of formula VII from cells and reacting the **protein** sample with iodoacetamide.
(II) acyl-NH-X-(epitope tag site)A-Y-(protease cleavage site)-Z-link; (III) acyl-NH-X-**alk**-O-Ph-CH2-Z-link; (VI) **protein**

-acyl-N-X-(epitope tag site)A-Y-(protease cleavage site)-Z-(lys- delta -N-iodoacetamide); (VII) **protein**-acyl-NH-X-(epitope tag site)A-Y-(protease cleavage site)-Z-Lys- delta -NHCOCH2.

A = an integer from 0-12;

X = an amide bond of formula -C(O)-NR-, a carbonyl of formula -C(O)-, or an amino acid sequence comprising between 0-50 amino acids, ;

Y = an amide bond of formula -C(O)-NR-, an amino acid sequence comprising between 0-50 amino acids;

Z = an amide bond of formula -(CH2)B-C(O)-NR-, an amide bond of formula -(CH2)B-NR-C(O)- or an amino acid sequence comprising between 0-10 amino acids;

R = hydrogen or lower alkyl;

B = an integer from 0-20;

alk = straight or branched chain of alkylene comprising between 0-20 carbon atoms;

Ph = a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the -CH2- group;

Link = -(CH2)C-I, -(CH2)D-CH(-(-CH2)ECH3)-(CH2F-X-I, Lys- epsilon -iodoacetamide, Arg- delta -iodoacetamide, or Orn- delta -iodoacetamide; and

C-F = an integer from 0-20.

An epitope tag site is a sequence of amino acids. When A is two or more, amino acid sequence of each epitope tag site can be the same or different, and protease cleavage site is a sequence of amino acids that is cleavage site for a highly specific protease enzyme.

USE - (C2) having a formula of (II) or (III) is useful for simultaneously identifying and determining the levels of expression of cysteine-containing **proteins** in normal and perturbed cells, which involves preparing a first **protein** sample or a first peptide sample from the normal cells, reacting the first **protein** sample or the first peptide sample with (C2), preparing a second **protein** sample or a second peptide sample from the perturbed cells, reacting the second **protein** sample or the second peptide sample with a second (C2), such that the molecular weight of the first reagent and the molecular weight of the second reagent are different by an integer multiple of 14 atomic mass units, combining the reacted first and second **protein** samples, subjecting the combined **protein** samples to proteolysis at a site on the **protein** samples or at a site on the peptide samples, the site being other than the protease cleavage sitem subjecting the proteolyzed combined **protein** samples to an **affinity chromatography** system comprising a second amino acid sequence attached to a solid, thereby forming bound **proteins** and non-bound **proteins**, where the epitope tag site of the reagent and the second amino acid sequence bind with high specificity to each other, eluting the non-bound **proteins** from the **affinity chromatography** system, subjecting the **affinity chromatography** system to a protease specific for the protease cleavage site, thereby forming a cleaved **protein** mixture, eluting the cleaved **protein** mixture from the **affinity chromatography** system, isolating the eluted **protein** mixture, subjecting the eluted **protein** mixture to **chromatographic separation**, followed by mass analysis, and comparing the results. The method optionally involves subjecting the prepared first **protein** sample or first peptide sample, and the prepared second **protein** sample or second peptide sample to proteolysis, and then reacting the proteolyzed first or second **protein** sample, or the proteolyzed first or second peptide sample with (C2) In the optional method, the link is Lys-

epsilon -iodoacetamide. Optionally, the first link is Orn- delta -iodoacetamide, and the second link is Lys- epsilon -iodoacetamide. The Z substituent in the first reagent has a molecular weight that is an integer multiple of 14 atomic mass units different than the Z substituent in the second reagent. The reagent reacts with the reactive side chain of one or more amino acid residues of a **protein** in the first or second **protein** sample. The amino acid residues are one of tyrosine, tryptophan, cysteine, methionine, proline, serine, threonine, lysine, histidine, arginine, aspartic acid, glutamic acid, **asparagine**, and glutamine. Most preferably, the amino acid residue is cysteine. The **chromatographic separation** is a multi-dimensional liquid **chromatographic separation**, preferably two- or three-dimensional liquid **chromatographic separation**.
The dimensions are any one size differentiation, charge differentiation, hydrophobicity, hydrophilicity or polarity. The mass analysis of step (n) is multi-dimensional, preferably two-dimensional mass analysis. (C2) is useful for proteomic analysis, which involves preparing a **protein** sample or a peptide sample from cells, reacting the **protein** sample or the peptide sample with (C2), subjecting the reacted **proteins** to proteolysis at a site on the **protein** samples, subjecting the proteolyzed reacted **proteins** or the proteolyzed reacted peptides to an **affinity chromatography** system comprising a second amino acid sequence attached to a solid support, thereby forming bound **proteins** and non-bound **proteins**, where the epitope tag site of the reagent and the second amino acid sequence bind with high specificity to each other, eluting the non-bound **proteins** from the **affinity chromatography** system, subjecting the **affinity chromatography** system to a protease specific for the protease cleavage site, thereby forming a cleaved **protein** mixture, eluting the cleaved **protein** mixture from the **affinity chromatography** system, subjecting the cleaved **protein** mixture to **chromatographic separation**, followed by mass analysis, comparing the results. (All claimed).

ADVANTAGE - The reagents allow rapid and quantitative analysis of **proteins** or **protein** function in mixtures of **proteins**. By preparing the reagent in two forms with detectably different masses, accurate relative quantification of peptide amounts using mass spectrometry, can be achieved.

Dwg.0/8

TECH UPTX: 20021007

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - Preferred Compound: The immobilization site is a solid surface comprising:
(a) a metal chelating column comprising nickel;
(b) an oligonucleotide, where the immobilization site is the complementary oligonucleotide; and
(c) magnetic residues, where the immobilization site comprises magnetic residues that bind magnetically to the magnetic residues of the solid surface.
Optionally, the immobilization site comprises amino acid residues. The immobilization site is preferably a direct link between the solid surface and the compound, the direct link being an acyl group, or a chemical moiety capable of reacting with the solid surface, thereby rendering the compound immobilized on the solid surface. The chemical moiety reacts reversible with the solid surface. The cleavage site is capable of breaking the molecule in two different parts, where one of the two different parts remains immobilized on the solid surface, while the other of the two different parts moves away from the solid surface by a wash

fluid. The cleavage site:
(a) may be an amino acid sequence, comprising at least one amino acid residue, where the amino acid sequence is a cleavage site for a protease;
(b) is a photocleavable linker that is cleaved heterolytically or homolytically when exposed to light of a certain wavelengths; or
(c) comprises a polynucleotide residue, of at least two nucleotides in length, and is cleaved with a restriction enzyme.
The cleavage site is a site that is capable of being cleaved. Preferably, the cleaving is by chemical cleavage that is carried out by adding acid or base. Optionally, the cleaving is by thermal cleavage, where the cleavage site comprises a polynucleotide residue that is capable of hybridizing to another polynucleotide residue connected to the immobilization site. In (C2), the protease cleave site comprises between 1-15 amino acids (preferably 4-8 amino acids). The protease cleavage site comprises at least 4 amino acids and comprises a sequence of Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly (S1). The protease enzyme is chosen from TEV protease, chymotrypsin, endoproteinase Arg-C, endoproteinase Asp-N, trypsin, Staphylococcus aureus protease, thermolysin or pepsin. The link is iodoacetamide coupled with a compound chosen from lysine, ornithine or arginine, and preferably comprises a sequence of (S1) or acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Lys-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Aps-Val-Pro-Aps-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Gly-Lys-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Ala-Lys-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-(GABA)-Lys-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Val-Lys-iodoacetamide, acyl-NH-Ala-Tyr-ProTyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Orn-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Gly-Orn-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Ala-Orn-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-(GABA)-Orn-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Val-Orn-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Arg-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Gly-Arg-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Ala-Arg-iodoacetamide, acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-(GABA)-Arg-iodoacetamide or acyl-NH-Ala-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Glu-**Asn**-Leu-Tyr-Phe-Gln-Gly-Val-Arg-iodoacetamide (S2-S16) (GABA -gamma-aminobutyric acid). Optionally, the link is a non-amino acid organic group. The link is preferably -(CH2)C-I or -(CH2)D-CH(-CH2)ECH3)-(CH2)F-X-I.
C, D, E, F = integers from 0-20.
Most preferably, the link is iodoacetamide, and is chosen from -CH(CH2C(O)I)CH2CH3, -C(C(O)ICH2CH2CH3, -CH(CH2I)CH2CH3, -CH2CH(CH2I)CH2CH2CH3. Preferably, **alk** is a straight or branched chain of alkylene having 0-20 carbon atoms, (preferably 0-3 carbon atoms). More preferably, **alk** is a straight chain alkylene chosen from methylene, ethylene, propylene, n-butylene, and n-pentylene. The substituents of Ph (Ia) are methoxy or nitro.
Z = an amino acid sequence comprising 1-3 amino acids, more preferably a single amino acid such as glycine, alanine or valine, or a synthetic amino acid containing an amino group at position other than alpha to the

carboxyl group e.g., beta, delta, epsilon, phi or gamma to the carboxyl group.
Most preferably, the Z is a gamma-aminobutyric acid.

L13 ANSWER 7 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-593209 [64] WPIDS
DNC C2002-167840
TI Forming array of antigens or antibodies, useful for **protein** analysis, comprises biotinylating fusion **protein** containing antigen or antibody binding **protein** and applying fusion **protein** to (strept)avidin coated non-porous support.
DC B04 **D16**
IN AUTON, K A
PA (NEXT-N) NEXTGEN SCI LTD
CYC 100
PI GB 2370039 A 20020619 (200264)* 52p
WO 2002081683 A2 20021017 (200270) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
GB 2370039 B 20021030 (200279)
ADT GB 2370039 A GB 2002-3448 20020214; WO 2002081683 A2 WO 2002-GB1623 20020404; GB 2370039 B GB 2002-3448 20020214
PRAI GB 2001-31025 20011228; GB 2001-8521 20010405
AB GB 2370039 A UPAB: 20021007
NOVELTY - Forming an array of antigens or antibodies (Ab) comprising:
(a) expressing in a recombinant cell, a fusion **protein** (FP) comprising an Ag or an Ab binding **protein** fused to a peptide;
(b) biotinylating the peptide of FP;
(c) applying the biotinylated peptide of the FP (bFP) to a(n) (strept)avidin coated non-porous support; and
(d) forming an array of at least 3 different **proteins** on the support, is new.
DETAILED DESCRIPTION - Forming (M1) an array of antigens (Ag) or antibodies (Ab) comprising:
(a) expressing in a recombinant cell, a fusion **protein** (FP) comprising an Ag or an Ab binding **protein** fused to a peptide (P1) with the sequence (S1) Leu-X1-X2-Ile-X3-X4-X5-X6-Lys-X7-X8-X9-10;
(b) biotinylating the peptide of the FP at the lysine residue adjacent to X6;
(c) isolating the biotinylated FP (bFP);
(d) applying the bFP to a(n) (strept)avidin coated non-porous support;
(e) forming an array of at least 3 different **proteins** on the support by either:
(i) where the FP comprising an Ag, carrying out steps (a)-(d) the desired number of times to form an Ag array; or
(ii) where the FP comprises an Ab binding **protein** applying to the **protein**, either before or after step (d), a number of different Ab or their binding fragments, where:
X1 = a naturally occurring amino acid;
X2 = any naturally occurring amino acid other than leucine, valine, isoleucine, tryptophan, phenylalanine or tyrosine;
X3 = phenylalanine or leucine;

X4 = glutamine or **asparagine**;
X5 = alanine, glycine, serine or threonine;
X6 = glycine or methionine;
X7 = isoleucine, methionine or valine;
X8 = glutamine, leucine, valine, tyrosine or isoleucine;
X9 = tryptophan, tyrosine, valine, phenylalanine, leucine or isoleucine; and
X10 = any naturally occurring amino acid other than **asparagine** or glutamine; where the peptide is capable of being biotinylated by a biotin ligase at the lysine residue adjacent to X6, is new.

INDEPENDENT CLAIMS are also included for the following:
(1) a **protein** array (I) obtained by M1;
(2) detecting (M2) binding between Ab and an Ag comprising: applying to (I) a sample which contains or is suspected of containing an Ab or an Ag; and detecting bound Ab or Ag on the support;
(3) a fusion **protein** comprising an Ab binding **protein** fused at the N- or C-terminus to S1; and
(4) a nucleic acid which encodes FP.

USE - The method and **proteins** are useful for producing arrays for conducting **protein** analysis, in particular of antibodies, antigens or antibody binding **proteins**.

ADVANTAGE - The fusion proteins allow the production of very good antigen or antibody arrays which can be efficiently produced on non-porous supports whilst substantially retaining the binding avidity of these proteins. By using a fusion of the antigen or antibody binding protein to P1, these proteins may be immobilized onto solid surfaces, whilst substantially maintaining the antigenicity of proteins or the binding capabilities of the antibody binding proteins. This may be because the fusion protein is biotinylated rather than the protein itself and so there is less disruption of the protein's antigenicity when attached to the support surface. In addition, P1 appears to reduce steric hindrance to enable interaction between antigen and antibody. By ensuring that the peptide linker is attached at a terminal region of the protein and contains the biotinylation site, sites on the protein which are essential for function appear to be largely unaffected. The biotinylated FP is immediately captured on application to the (strept)avidin coated support leading to very discrete spots of protein with minimal observable diffusion.

Dwg.0/15

TECH UPTX: 20021007

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In M1, the recombinant cell expresses biotin ligase and biotin and step (b) is effected in the presence of biotin such that biotinylation occurs in vivo in the cell. Step (c) is effected using a further Ab or its binding fragment, which is specific for P1 or P2. The further Ab or its binding fragment is immobilized on a column, magnetic bead or loaded into a pipette tip. The bound FP is eluted by increasing the pH conditions. The FP is isolated using a **separation** material (**modified** version of (strept)avidin which has a lower **affinity** than native (e.g. strept)avidin attached to magnetic beads or pipette tips) which releasably binds biotin. Some areas of the coated support are blocked to prevent binding of FP. Prior to step (d), the identity of FP is confirmed using mass spectroscopy. **Protein** normalization is carried out by detecting P1 or P2 in the FP which acts as an internal control. Detection is by an Ab with a high **affinity** for the peptide.

Protein normalization is effected by performing an immunoassay simultaneously with subsequent analysis of a biological sample using the

array. The non-porous support is glass or plastic material. A further acceptor layer is provided on top of the foundation of the streptavidin later on the support. The array comprises 3-10,000 different FP. In M2 detection is via enzyme linked immunosorbant assay (ELISA). The FP array continues to be monitored for quality and/or density of the **protein** by detecting P1 or P2. At least some of the steps, preferably all are operated automatically.
Preferred **Protein**: FP further comprises a second peptide sequence (P2) capable of acting as an **affinity** or detection tag sequence to FP where the sequence comprises between 1-30 amino acids. P2 is fused to the end of S1 not fused to Ag or Ab binding **protein**.
At least one amino acid of P2 is histidine. P2 has the formula His-X or Y-His, where:
X = Gly, His, Tyr, Trp, Val, Leu, Ser, Lys, Phe, Met, Ala, Glu, Ile, Thr, Asp, **Asn**, Gln, Arg, Cys or Pro; and
Y = Gly, Ala, His or Tyr.
S1 is selected from one of 71 fully defined sequences comprising 15 amino acids as given in the specification. P1 comprises a fully defined sequence (S2) of Gly-Leu-**Asn**-Asp-Ile-Phe-Glu-Ala-Gln-Lys-Ile-Glu-Trp-His-Glu. FP comprises an Ag. An Ag library is used to create the array. The Ab binding **protein** is a mixture of **Protein** A, **Protein** G and **Protein** L.
Preferred Nucleic Acid: The nucleic acid comprises the sequence GGCCTGAACGACATCTTCGAGGCTCAGAAAATCGAATGGCACGAA.

L13 ANSWER 8 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-304072 [34] WPIDS
CR 2002-089807 [12]
DNN N2002-237932 DNC C2002-088421
TI Detecting multiple analytes by separating a set of reporter signals having common property from molecules lacking common property, altering signal, detecting and distinguishing altered forms of signal from each other.
DC B04 **D16** S03
IN CHAIT, B T; KERSHNAR, E R; LATIMER, D R; LIZARDI, P M; MATTESSICH, M J; MCCONNELL, K J; MORROW, J S; ROTH, M E; MCCONNEL, K J
PA (AGIL-N) AGILIX CORP; (CHAI-I) CHAIT B T; (KERS-I) KERSHNAR E R; (LATI-I) LATIMER D R; (LIZA-I) LIZARDI P M; (MATT-I) MATTESSICH M J; (MCCO-I) MCCONNELL K J; (MORR-I) MORROW J S; (ROTH-I) ROTH M E
CYC 96
PI WO 2002014867 A2 20020221 (200234)* EN 341p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001083562 A 20020225 (200245)
US 2003045694 A1 20030306 (200320)
ADT WO 2002014867 A2 WO 2001-US41709 20010813; AU 2001083562 A AU 2001-83562 20010813; US 2003045694 A1 Provisional US 2000-224939P 20000811, Provisional US 2001-283498P 20010412, US 2001-929266 20010813
FDT AU 2001083562 A Based on WO 200214867
PRAI US 2001-283498P 20010412; US 2000-224939P 20000811; US 2001-929266 20010813
AB WO 200214867 A UPAB: 20030324
NOVELTY - Detecting (M1) multiple analytes involves separating a set of reporter signals (RS), where each RS has a common property, from molecules lacking the common property, altering the RS, and detecting and

distinguishing the altered forms of the RS from each other.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a set of RS (I) comprising a number of RS, where the RS have a common property, allowing the RS to be distinguished or separated from molecules lacking this property, the RS can be altered, and be distinguished from every other altered form of RS;

(2) kits comprising a set of reporter molecules (RM), where

(a) each RM comprises an RS and a decoding tag, where each RM comprises a different decoding tag and RS, and a set of coding molecules comprising a specific binding molecule interacting with a different analyte, and coding tag interacting with a different decoding tag; or

(b) or an RS and a coupling tag, where the RM comprises a different coupling tag and different RS;

(3) a set of labeled **proteins** (LPs) (II) where each LP comprises a **protein** or peptide and RS attached to the **protein** or peptide, where alteration of the RS alters the LPs, and altered forms of each LP can be distinguished from every other altered form of LP;

(4) a labeled **protein** (III) where the LP comprises a **protein** or peptide and a RS attached to the **protein** or peptide, where the LP has a common property, and the common property allows the LP to be distinguished or separated from molecules lacking the common property, the RS can be altered, and alteration of the RS alters the LP, where altered form of the LP can be distinguished from the unaltered form of LP;

(5) detecting (M2) a **protein**, by detecting a LP, where the LP comprises a **protein** or peptide and a RS attached to the **protein** or peptide, where the LP is altered by altering the RS, detecting an altered form of the LP, where the LP is altered by altering the RS, and identifying the **protein** based on the characteristics of the LP and altered form of the LP;

(6) a catalog (IV) of **proteins** and peptides comprises **proteins** and peptides in a sample detected by M1;

(7) producing (M3) a **protein** signature;

(8) analyzing (M3) a **protein** sample, by mixing a **protein** sample with a predetermined amount of RS calibrator, where the **protein** sample has a known amount of **protein** and comprises a target **protein** fragment, the target **protein** fragment and the RS calibrator can be altered, where the altered form of the RS calibrator can be distinguished from the unaltered form of the target **protein** fragment, altering the target **protein** fragment and RS calibrator, and detecting the altered forms of the target **protein** fragment and RS calibrator;

(9) a set of RS calibrators (V);

(10) a kit for producing a **protein** signature comprising (V) and one or more reagents for treating a **protein** sample to produce **protein** fragments;

(11) a mixture (VI) comprising (V) and a set of target **protein** fragments;

(12) a set of target **protein** fragments (VII);

(13) a set of nucleic acid molecules (VIII) where each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a **protein** or peptide of interest;

(14) a set of amino acid segments (IX) where each amino acid segment comprises a RS peptide and a **protein** or peptide of interest;

(15) a cell (X) comprising (VIII);

(16) a set of cells (XI) comprising a nucleic acid molecule where

each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a **protein** or peptide of interest;

(17) an organism (XII) comprising (VIII);

(18) a set of organisms (XIII) each organism comprising a nucleic acid molecule where each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a **protein** or peptide of interest;

(19) detecting (M4) expression involves detecting a target altered RS peptide derived from one or more expression samples, where the expression samples collectively comprise (VIII);

(20) detecting (M5) cells involves detecting a target altered RS peptide derived from one or more (X);

(21) detecting (M6) cell samples by detecting a target altered RS peptide derived from one or more cell samples; and

(22) detecting (M7) organisms by detecting a target altered RS peptide derived from one or more (XII).

USE - The method (M1) is useful for detecting multiple analytes (claimed). M1 is useful for detection of analytes and biomolecules, (such as **proteins**, peptides and **protein** fragments), preferably for multiplex detection and analysis of analytes and biomolecules. M1 is useful to detect a specific analyte (in a specific sample or in multiple samples) or multiple analytes (in a single sample or multiple samples), and to gather and catalog information about unknown analytes. M1 is useful as a detection system in a number of fields, including antibody or **protein** microarrays, DNA microarrays, expression profiling, comparative genomics, immunology, diagnostic assay and quality control. M1 is useful as a detection and analysis system for **protein** analysis, proteome analysis, proteomic, **protein** expression profiling, de novo **protein** discovery, functional genomics and **protein** detection.

ADVANTAGE - M1 increases the sensitivity and accuracy of detection of analytes of interest, and allows a complex sample of analytes to be quickly and easily cataloged in a reproducible manner. M1 is compatible with techniques involving cleavage, treatment or fragmentation of a bulk sample in order to simplify the sample prior to introduction into the first stage of a multistage detection system. M1 is also compatible with any desired sample, including raw extracts and fractionated samples.
Dwg.0/10

TECH UPTX: 20020528

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Methods: M1 involves **separating** one or more labeled **proteins** (LP), where each LP comprises a **protein** or peptide and RS attached to **protein** or peptide, **altering** RS, thus **altering** LP, and detecting and distinguishing the **altered** forms of LP from each other. When associating one of a number of RS with one or more analytes in each of a number of samples the analytes are **separated** by contact with a capture array. Associating one of a number of RS with one or more analytes in each of a number of samples to form RS/analyte conjugates, having a common property, comprises **separating** the analytes (not based on the common property), **altering** the RS and RS/analyte conjugates and detecting the **altered** forms of the RS/analyte conjugates. The different sets of RS can be associated with different samples, can comprises the same RS and contain a single RS. Associating a number of coding tags (oligonucleotides), where the RS are peptide nucleic acids, with one or more analytes in at least one sample comprises: associating the coding tags with one or more RS molecules comprising a reporter signal and a decoding tag;

altering the RS; and detecting the **altered** forms of the reporter molecules. The common property is mass-to-charge ratio, and RS are **altered** by **altering** their mass, where the **altered** forms of RS can be distinguished by differences in the mass-to-charge ratio of the **altered** forms of RS. The mass of the reporter signals can also be **altered** by fragmentation. The sample can be produced by standard **separation** procedures, and can be the same or different. In M1 not all the RS are **altered,** and they are not all detected at the same time, or all the RS are **altered** and all **altered** forms of RS are detected at different times. The **altered** forms of the target **protein** fragment can be detected by mass spectrometry, preferably a tandem mass spectrometer comprising a first stage quadrupole mass filter and a final stage time of flight analyzer.

Treating (M3) a **protein** sample to produce **protein** fragments, where the **protein** fragments comprise a set of target **protein** fragments which can be **altered,** and the **altered** forms of the target **protein** fragments can be distinguished from the other **altered** forms of the target **protein** fragments comprises;

(a) mixing the target fragments with a set of RS calibrators, where each target **protein** fragment shares a common property with at least one of the RS calibrators, where the common properties allow the target **protein** fragments and RS calibrators having the common property to be distinguished or **separated** from molecules lacking the common property, and the target **protein** fragment and RS calibrator that share a common property correspond to each other;

(b) **separating** the target **protein** fragments and RS calibrators from other molecules based on the common properties of the target **protein** fragments and RS calibrators;

(c) **altering** the target **protein** fragments and RS calibrators; and

(d) detecting the **altered** forms of the target **protein** fragments and RS calibrators, where the presence, absence, amount or presence and amount of the **altered** forms of the target **protein** fragments indicates the presence, absence, amount, or presence and amount in the **protein** sample of the target **protein** fragments from which the **altered** forms of the target **protein** fragments are derived, where the presence, absence, amount or presence and amount of the target **protein** fragments in the **protein** sample constitutes a **protein** signature of the **protein** sample.

The amount of at least two RS calibrators can be different or the same. The **protein** fragments are produced by serine protease digestion, e.g., trypsin or with Factor Xa or enterokinase. One of the target **protein** fragments comprises at least one **modified** amino acid e.g., phosphorylated, acylated or glycosylated. M3 is also carried out on a tester sample which is treated to destroy one or more **proteins** that can still be produced from the control **protein** sample. A second type of **protein** signature can be produced from the same or different cell types, where the **protein** signature indicates the physiological state of the cells. The cells are preferably human.

M4 comprises treating an organism, preferably a human, by administering a compound. It can also further comprise mixing of two or more expression samples together. Expression samples are produced by lysing the cells, and can be produced from different organisms. The peptide of interest comprises at least two amino acid segments that can be the same or

different, are **proteins** in the same enzymatic pathway, or can be **proteins** associated with the same disease. The nucleotide segment encodes a number of amino acid segments each comprising a reporter signal peptide and a **protein** or peptide of interest, comprising a single nucleic acid molecule comprising a number of nucleotide segments each encoding an amino acid segment. The amino acid segment comprises a trypsin cleavage site. Detection of an **altered** reporter signal peptide indicates expression in the expression sample comprising the reporter signal peptide, from which the detected **altered** reporter signal peptide is derived. The amount of nucleotide segment present is proportional to the amount of the target **altered** reporter signal peptide detected.
M5 comprises cells having a trait of interest comprising the same RS peptide i.e., a heterologous gene encoding an amino acid segment. Detection of the target **altered** RS indicates the presence of the cell sample from which the target **altered** RS peptide is derived.
In M7 the organism comprises a transgene encoding the amino acid segment comprising the RS peptide and **protein** of interest , where the RS peptide in each organism is different.
Preferred Reporter Signal: The **alteration** of RS also **alters** their charge. The RS are **altered** by cleavage at a photocleavable amino acid or fragmented in a collision cell or at an **asparagine**-proline bond. The set comprises 2 or more, preferably 100 or more different reporter signals such as peptide, oligonucleotide, carbohydrates, polymers, oligopeptides or peptide nucleic acids. When the RS comprises peptides they can have the same or different amino acid composition, a labile or scissile bond in a different location, or contain a different distribution of heavy isotopes. The common property is not an **affinity** tag, and one or more **affinity** tags are associated with the RS.
(V111) comprises transcription/translation expression sequences where the amino acid segment can be expressed in vitro, in vivo, and in cell culture. The expression sequences may be differentially regulated and may be genes expressed by part of the same expression cascade. (V111) can further comprise replication sequences, integration sequences and be replicated using pairs of primers. Each first primer also encodes an epitope tag. The RS peptide of each amino acid sequence can be the same or different. (V111) are contained in substantially all the cells of an organism and can be integrated into artificial organisms. The amino acid segment comprises a self-cleaving intein segment.

L13 ANSWER 9 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-179782 [23] WPIDS
DNC C2002-055891
TI Vaccine composition for treating cancer, in particular lung cancer, autoimmune diseases and other related conditions, comprises a lung tumor associated antigen, especially CASB761 polypeptide.
DC B04 **D16**
IN CASSART, J; GAULIS, S; VINALS Y DE BASSOLS, C
PA (SMIK) SMITHKLINE BEECHAM BIOLOGICALS
CYC 96
PI WO 2002006338 A1 20020124 (200223)* EN 92p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2001076392 A 20020130 (200236)
ADT WO 2002006338 A1 WO 2001-EP7967 20010711; AU 2001076392 A AU 2001-76392 20010711
FDT AU 2001076392 A Based on WO 200206338
PRAI GB 2000-17512 20000717
AB WO 200206338 A UPAB: 20020411
NOVELTY - A vaccine composition (V1) comprising a polypeptide with an amino acid sequence which has 85 % identity to a CASB761 polypeptide having a sequence of 238 (S1-human), 233 (S2-rat) or 231 (S3-mouse) amino acids, given in the specification (or its immunogenic fragment), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a vaccine composition (V2) comprising a polynucleotide which comprises a nucleotide sequence having 85 % identity with a nucleotide sequence (S4) of 1771 base pairs (bp), given in the specification, or its fragment which encodes an immunogenic polypeptide;

(2) a vaccine composition (V3) comprising antigen presenting cells, **modified** by in vitro loading with a CASB761 polypeptide, or genetically **modified** in vitro to express the polypeptide;

(3) a polypeptide (I) comprising an immunogenic fragment of a CASB761 polypeptide in which the immunogenic activity of the fragment is the same as the polypeptide having the sequence (S1 - S3);

(4) an immunogenic fragment (II) of human CASB761 polypeptide comprising a sequence chosen from 45 sequences of defined amino acids given in the specification such as (F1) - (F5);

(5) an isolated polynucleotide (III) encoding (I) or (II);

(6) an expression vector (IV) comprising (III);

(7) a recombinant live microorganism (V) comprising (III);

(8) a host cell (VI) comprising (III), (IV) or (V);

(9) producing an immunogenic composition by admixing CASB761 polypeptide, its immunogenic fragment or fusion polypeptide, or CASB761 polynucleotide, with a suitable adjuvant, diluent or carrier;

(10) producing (I) or (II);

(11) an antibody (VII) immunospecific for (I) or (II);

(12) inducing (M1) an immune response against a human CASB761 polypeptide in a human, by administering a composition comprising a xenogeneic form of the human polypeptide or polynucleotide;

(13) treating (M2) a subject by immunoprophylaxis or therapy, by in vitro induction of immune response to a molecule of any of (S1) - (S4), using in vitro incubation of the polypeptide or polynucleotide comprising (S1) - (S4), with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for treating the disease;

(14) screening to identify compounds that stimulate or inhibit the function or level of human CASB761 polypeptide, by:

(a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or its fusion **protein** by using a label directly or indirectly associated with the candidate compound, or in the presence of a labeled competitor;

(b) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes expressing the polypeptide;

(c) mixing a candidate compound with a solution containing a human CASB761 polypeptide, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(d) detecting the effect of a candidate compound on the production of mRNA encoding the polypeptide and the polypeptide in cells, using an

enzyme linked immunosorbant assay (ELISA) assay;

(15) an agonist or antagonist to the polypeptide having the sequence (S1);

(16) a compound which is agonist or antagonist to a human CASB761 polypeptide, or a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide, for use in therapy; and

(17) diagnosing (M3) a disease or a susceptibility to a disease in a subject related to expression or activity of CASB761 polypeptide or polynucleotide, in a subject, by analyzing the presence or amount of polypeptide or polynucleotide in a sample derived from the subject.

Gln Leu Leu Asp Glu His Asp Ala Val (F1)
Lys Leu Val **Asn** Leu Gly Phe Ala Thr (F2)
Glu Tyr Ile Arg Ala Leu Gln Gln Leu (F3)
His Val Pro **Asn** Gly Ala Ala **Asn** Lys (F4)
Lys Arg Arg Leu **Asn** Phe Ser Gly Phe (F5)

ACTIVITY - Cytostatic; immunosuppressive. Tests are described but no results are given.

MECHANISM OF ACTION - Vaccine; gene therapy.

USE - (I) and a polynucleotide (III) encoding (I) are useful in the manufacture of a vaccine for immunotherapeutically treating a patient suffering from or susceptible to lung cancer, lung preneoplastic lesions or other related conditions. Treating (M2) a subject by immunoprophylaxis or therapy is useful for treating lung cancer, and diagnosing (M3) a disease or a susceptibility to a disease is useful for diagnosing the presence of lung cancer or susceptibility to lung cancer in a subject. Vaccines (V1 - V3) are useful in medicine (claimed), for treating cancer, particularly lung cancer, autoimmune diseases and other related conditions. CASB761 polynucleotides and their portions are useful as diagnostic reagents, to diagnose different forms and states of cancer, in staging cancerous disorder and grading the nature of the cancerous tissue. An antibody (VII) is useful to isolate and to identify clones expressing CASB761 polypeptide or to purify the polypeptide by affinity chromatography and to treat or prevent, particularly lung cancer, autoimmune disease and related conditions.

Dwg.0/2

TECH UPTX: 20020411

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) is produced by culturing (VI) under conditions sufficient for the production of the polypeptide and recovering the polypeptide from the culture medium (claimed).

Preferred Composition: The vaccine composition additionally comprises a TH-1 inducing adjuvant such as 3D-MPL, QS21, a mixture of QS21 and cholesterol, a CpG oligonucleotide or a mixture of two or more adjuvants. The amino acid sequence has at least 95 % identity with (S1 - S4) or its immunogenic fragment.

Preferred Polypeptide: (I) or (II) is a part of a larger fusion **protein**, and is chemically conjugated to a carrier **protein**.

Preferred Method: In (M1), the xenogeneic polypeptide or polynucleotide is isolated from mouse or rat.

L13 ANSWER 10 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2002-033730 [04] WPIDS
DNC C2002-009355
TI Genetically-encodable, environmentally-responsive fusion **proteins** comprising Elastin-Like peptides polypeptides.
DC B04 **D16**
IN CHILKOTI, A

PA (CHIL-I) CHILKOTI A
CYC 95
PI US 2001034050 A1 20011025 (200204)* 57p
WO 2002074928 A2 20020926 (200273) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
ADT US 2001034050 A1 Provisional US 2000-190659P 20000320, US 2001-812382 20010320; WO 2002074928 A2 WO 2002-US8523 20020320
PRAI US 2000-190659P 20000320; US 2001-812382 20010320
AB US2001034050 A UPAB: 20020117
NOVELTY - Genetically-encodable, environmentally-responsive fusion **proteins** comprising Elastin-Like peptides (ELP) polypeptides, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a fusion **protein** (I) exhibiting a phase transition, comprising:

(a) 1 or more biological molecules;

(b) 1 or more **proteins** exhibiting a phase transition joined to the biologically active molecule; and

(c) optionally a spacer sequence separating (a) and/or (b);

(2) a polynucleotide (II) encoding (I);

(3) an expression vector (III) comprising (II);

(4) a host cell (IV) transformed by the expression vector (III) which expresses the fusion **protein**;

(5) a method (V) of producing one or more fusion **proteins** comprising:

(a) transforming a host cell with the expression vector (III); and

(b) causing the host cell to express the fusion **protein**;

(6) a method (VI) for isolating one or more fusion **proteins** comprising:

(a) expressing the fusion **protein**(s) via (V);

(b) disrupting the cells to release the fusion **proteins**; and

(c) isolating the **proteins** by a method comprising raising temperature;

(7) a method (VII) for isolating one or more fusion **proteins** comprising:

(a) expressing the fusion **proteins** via (V);

(b) isolating the **proteins** by raising temperature;

(8) a method (VIII) of optimizing size of an ELP expression tag incorporated in a polynucleotide comprising a nucleotide sequence encoding a fusion **protein** exhibiting a phase transition (the fusion **protein** comprises a **protein** of interest), comprising:

(i) forming a number of polynucleotides comprising a nucleotide sequence encoding a fusion **protein** exhibiting a phase transition (each of the polynucleotides includes a different-sized ELP expression tag);

(ii) expressing corresponding fusion **proteins** from the polynucleotides;

(iii) determining a yield of the desired **protein** for each of the corresponding fusion **proteins**;

(iv) determining size of particulates for each of the corresponding fusion **proteins** in solution as temperature is raised above Tt;

and

(v) selecting an optimized size ELP expression tag according to pre-determined selection criteria for maximum recoverable **protein** of interest from among the polynucleotides;

(9) a method (IX) of purification of fusion **proteins** to yield a **protein** of interest, comprising forming a polynucleotide comprising a nucleotide sequence encoding a fusion **protein** exhibiting a phase transition, expressing the fusion **protein** in culture, and subjecting a fusion **protein**-containing material from the culture to processing involving centrifugation and inverse transition cycling to recover the **protein** of interest.

USE - The polypeptides may be used in medicine and biotechnology.

ADVANTAGE - The fusion **proteins** exhibit unique physico-chemical and functional properties that can be modulated as a function of the solution environment. The invention also provides methods for purifying the ELPs, which take advantage of these unique properties, including high-throughput purification methods that produce high yields (e.g., milligram levels) of purified **proteins**, therefore yielding sufficient purified product for multiple assays and analyses. The high throughput purification technique is simpler and less expensive than current commercial high throughput purification methods, since it requires only one transfer of purification intermediates to a new multiwell plate.

Dwg.0/30

TECH UPTX: 20020117

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Fusion **Proteins**: In the fusion **protein** (I) the biological molecule comprises a therapeutic **protein**, an enzyme useful in industrial biocatalysis, or comprises an antibody or antibody fragment with **affinity** for a **protein** of interest (and upon binding to the **protein** of interest, the fusion **protein** retains some or all of its phase transition character).

The phase transition is mediated by 1 or more methods selected from:

(a) changing temperature (preferably raising the temperature);

(b) changing pH;

(c) addition of organic solutes and/or solvents;

(d) side-chain ionization or chemical **modification**; and

(e) changing pressure.

The **protein**(s) of (b) exhibit a beta-turn. The **protein** of (b) comprises oligomeric repeats of the pentapeptide:

Val-Pro-Gly-X-Gly

X = any natural or non-natural amino acid residue (X optionally varies among oligomeric repeats).

The X component(s) of the oligomeric repeats comprise(s) a naturally-occurring amino acid residue. The X component(s) of the oligomeric repeats comprise(s) 1 or more amino acid residues selected from: alanine, arginine, **asparagine**, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, seine, threonine, tryptophan, tyrosine and valine residues.

Any two or more of the oligomeric repeats are **separated** by one or more amino acid residues which do not eliminate the phase transition characteristic of the fusion **protein**. The ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 75%, preferably greater than about 95%.

The fusion **protein** (I) comprises the spacer sequence of (c), which comprises a proteolytic cleavage site. The fusion **protein** further comprises a signal peptide which is cleavable from the fusion **protein** by enzymatic cleavage. The signal peptide directs

secretion of the fusion **protein** from the cell. The **protein**(s) exhibiting the phase transition are beta-turn **proteins** joined at the C- and/or N-terminus of a **protein** of (a).
Preferred Methods: In (V) the expressed fusion **protein** comprises a signal sequence directing secretion of the fusion **protein** from the cell. The method comprises expressing the fusion **protein** in culture in a well of a microplate and/or processing the fusion **protein**-containing material from the said culture in a well of a microplate.
Preparation: The fusion **protein** (I), the biological molecule(s) of (a), **protein**(s) of (b) and spacer sequence of (c) (when present) are recombinantly or synthetically produced.

L13 ANSWER 11 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2001-488881 [53] WPIDS
DNC C2001-146834
TI New product for treating thrombosis, comprises a dendroaspin scaffold in which a native motif has been deleted or replaced by an amino acid sequence with or without integrin-binding activity.
DC B04 **D16**
IN KAKKAR, V V; LU, X
PA (TRIG-N) TRIGEN LTD; (KAKK-I) KAKKAR V V; (LUXX-I) LU X
CYC 95
PI WO 2001057210 A2 20010809 (200153)* EN 39p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001028714 A 20010814 (200173)
US 2002120102 A1 20020829 (200259)
EP 1252313 A2 20021030 (200279) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
ADT WO 2001057210 A2 WO 2001-GB439 20010205; AU 2001028714 A AU 2001-28714 20010205; US 2002120102 A1 US 2001-779054 20010205; EP 1252313 A2 EP 2001-949004 20010205, WO 2001-GB439 20010205
FDT AU 2001028714 A Based on WO 200157210; EP 1252313 A2 Based on WO 200157210
PRAI GB 2000-2625 20000205
AB WO 200157210 A UPAB: 20010919
NOVELTY - A product (I) comprising a dendroaspin scaffold in which the native Arg-Gly-Asp motif has been deleted or has been replaced by a replacement amino acid sequence which is an amino acid sequence having no integrin-binding activity or an integrin-binding amino acid sequence and comprising a tripeptide sequence other than Arg-Gly-Asp containing Asp or Glu adjacent to Gly, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a nucleic acid molecule (II) encoding (I);
(2) a plasmid (III) comprising (II);
(3) a host cell (IV) transformed with (III);
(4) a cell culture (V) comprising (IV);
(5) producing (I); and
(6) a pharmaceutical composition (VI) comprising (I).
ACTIVITY - Antitumor; cardiant; thrombolytic. No biological data is given.

MECHANISM OF ACTION - None given.

USE - (I) is useful as a pharmaceutical, and for the manufacture of a medicament for the treatment or prophylaxis of disease associated with thrombosis, myocardial infarction, retinal neovascularization, and endothelial injury in human or animal patient. (I) is also useful for investigating function, effects, or activity of one or more non-wild-type dendroaspin sequences contained in (I). (I) is useful for investigating the function, effects or activity of a species other than a wild-type dendroaspin sequence, by providing (I) which comprises the species and performing in vivo or in vitro tests with (I), and formulating (I) into a medicament. The dendroaspin is useful as a scaffold for one or more non-dendroaspin amino acid sequences in a dendroaspin framework in which the native Arg Gly Asp motif has been deleted or has been replaced by a replacement amino acid sequence which is an amino acid sequence having non integrin-binding activity or an aspartic acid- or glutamic acid-containing integrin-binding amino acid sequence other than Arg Gly Asp (claimed). (I) is useful as a vehicle for non-dendroaspin domains, for presenting an amino acid sequence to a target for experimental purposes, as scientific tools, and for developing active agents, especially for pharmaceutical purposes or to obtain information useful in the development of small molecule therapeutic or diagnostic agents. (I) is useful for treating dysregulated apoptosis, abnormal cell migration, leukocyte recruitment, immune system activation, tissue fibrosis and tumorigenesis.

ADVANTAGE - (I) forms a stable vehicle for non-dendroaspin groups irrespective of whether the modified scaffold retains the Arg Gly Asp sequence or any integrin-binding activity.

Dwg.0/1

TECH UPTX: 20010919

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) is prepared by:

(1) culturing (IV) to express (I), extracting (I) from the culture and purifying it; or

(2) preparing an expression vector comprising a nucleic acid sequence encoding (I) operatively linked to a promoter and optionally linked to a nucleic acid sequence encoding a heterologous **protein** for co-expression, transforming a host cell with the vector and causing the host cell to express the **modified** dendroaspin nucleic acid sequence.

The expression vector is prepared by assembling from overlapping oligonucleotides the coding sequence of a dendroaspin scaffold containing a Arg Gly Asp motif and operatively linking the resulting cDNA to a promoter, where the promoter is optionally linked to a nucleic acid sequence encoding a heterologous **protein** for expression of fusion **protein**, and **modifying** the Arg Gly Asp-encoding domain of the expression vector to encode (I). The method further comprises before or after **modification**, **modifying** at least one other domain of the nucleic acid sequence of the vector encoding the dendroaspin scaffold by one or more of the insertion, deletion, or substitution of nucleic acid residues so that on expression the dendroaspin scaffold comprises a corresponding domain having a non-wild type dendroaspin sequence. Preparation of the expression vector comprises constructing from oligonucleotides an expression vector comprising a nucleic acid sequence encoding (I), and, optionally, **modifying** at least one other domain of the nucleic acid sequence of the vector encoding the dendroaspin scaffold by one or more insertion, deletion or substitution of nucleic acid residues so that on expression the dendroaspin scaffold comprises a corresponding domain having a non-wild-type dendroaspin sequence. The method further comprises extracting the **modified** dendroaspin from a host cell culture,

purifying the **modified** dendroaspin from the cell culture extract, and if the **modified** dendroaspin is a fusion **protein**, cleaving the dendroaspin portion from the heterologous portion of the fusion **protein**. The heterologous **protein** is glutathione S-transferase (GST) and the purification involves GST **affinity chromatography** followed by cleavage of the **modified** dendroaspin from GST (claimed).

Preferred Product: In (I), the tripeptide sequence is of the formula B-J-Z, where J-Z is Gly Asp or Gly Glu and B is Arg, Lys, Gln, Ala, His, **Asn**, Val, Ile, Leu, Met, Phe, Pro or Trp, B-J is Asp Gly or Glu Gly and Z is any amino acid, or J is Asp or Glu and B and Z are each independently selected from Ala, Val, Ile, Leu, Met, Phe, Pro or Trp. B is Arg, Lys, Gln, Ala, His or **Asn** provided that B-J-Z in not Arg Gly Asp. Z is preferably Glu, Arg or Pro. B-J-Z is bonded at its C-terminal end to Met, Trp, **Asn** or Val. Met, Trp, **Asn** or Val residue is followed by Pro which is at position 47 of wild type dendroaspin or by an Ala residue substituted for it. The integrin-binding amino acid sequence is preceded by the Pro which is at position 42 of wild type dendroaspin or by an Ala residue substituted for it. Z is followed by Pro which is at 47 of wild type dendroaspin or by a Ala inserted before the wild type position 47 Pro. (I) further comprises the replacement amino acid sequence having no integrin binding activity and having a receptor binding function. The replacement amino acid sequence in its native polypeptide enters the pocket to function. (I), in addition to deletion or replacement of the Arg Gly Asp motif, comprises at least one foreign (non-wild-type dendroaspin) domain which comprises at least one non-dendroaspin domain which confers functionality on the product. (I) comprises at least two non-wild-type dendroaspin domains optionally having the same sequence, where the domain has two or more amino acid sequence portions **separated** by at least one amino acid residue of dendroaspin. (I) contains a non-wild-type dendroaspin domain conferring platelet derived growth factor (PDGF) activity, glycoprotein IBalpha activity, hirudin activity, thrombomodulin activity, vascular epidermal growth factor activity, transforming growth factor-beta1 activity, blast fibroblast growth factor activity, angiotensin II activity, factor VIII activity, von-Willebrand factor activity, tick anticoagulant **protein** (TAP) activity or nematode anticoagulant **protein** (NAP) activity. The non-wild-type dendroaspin domain is derived from the above mentioned factors or a sequence having homology to at least part of such sequence. The non-wild-type domains is/are incorporated into loop I and/or loop II, loop I and/or loop III, loop II and/or loop III, or loop I, loop II and loop III of the dendroaspin scaffold. The non-wild-type domain is preferably a single non-wild-type domain and is incorporated into either loop I or loop II. The non-wild-type domains is/are contained in the dendroaspin scaffold between amino acid residues selected form one or more of 4-16, 18-21, 22-36, or at the end of the dendroaspin scaffold after residue 50. (I) comprises a dendroaspin scaffold in which the Arg Gly Asp motif has been replaced by a integrin-binding amino acid sequence at one domain and a non-dendroaspin species which confers a second functionality on another domain. The residues of a non-loop region of the dendroaspin scaffold are augmented by the non-dendroaspin species which confers a second functionality on another domain. The loop III is additionally **modified** as compared with native dendroaspin by insertion, deletion or substitution of one or more amino acid residues. A maximum of 8 and a minimum of 1 amino acids **modified** by additional **modification** within loop III. Arg Gly Asp is replaced by the integrin binding sequence and the additional **modification** comprises **modification** of the amino acids flanking integrin

binding sequence. The loop I and/or loop II are additionally **modified** by insertion, deletion or substitution of one or more amino acids. (I) contains not more than 100, preferably 14-36 amino acid residues more than native dendroaspin.
Preferred Polynucleotide: (II) is operatively linked to a promoter and optionally to a nucleic acid sequence encoding a heterologous **protein** or its peptide thereby to encode a fusion product. The promoter is isopropyl b-D-thiogalactopyranoside (IPTG) inducible and optionally the heterologous **protein** or peptide is glutathione S-transferase.
Preferred Plasmid: (III) is preferably plasmid pGEX-3X.
Preferred Host Cell: (IV) is Escherichia coli.

L13 ANSWER 12 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2001-282040 [29] WPIDS
DNN N2001-201000 DNC C2001-085973
TI New identification peptide containing many linked copies of an antigen domain, useful for purification and detection of recombinant fusion **proteins**.
DC B04 **D16** S03
IN BRIZZARD, B L; HERNAN, R; BRIZZARD, B
PA (SIGM-N) SIGMA-ALDRICH CO
CYC 95
PI WO 2001027293 A1 20010419 (200129)* EN 51p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000077049 A 20010423 (200147)
US 2002045193 A1 20020418 (200228)
US 6379903 B1 20020430 (200235)
EP 1220933 A1 20020710 (200253) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT WO 2001027293 A1 WO 2000-US25693 20000920; AU 2000077049 A AU 2000-77049 20000920; US 2002045193 A1 Div ex US 1999-415000 19991008, US 2001-970308 20011003; US 6379903 B1 US 1999-415000 19991008; EP 1220933 A1 EP 2000-966755 20000920, WO 2000-US25693 20000920
FDT AU 2000077049 A Based on WO 200127293; EP 1220933 A1 Based on WO 200127293
PRAI US 1999-415000 19991008; US 2001-970308 20011003
AB WO 200127293 A UPAB: 20010528
NOVELTY - Identification polypeptide (IP) for purifying a target peptide (TP) comprises many copies, linked in tandem, of an antigenic domain (AD) containing no more than 20 amino acids (aa) and a linking sequence (LS) between the many copies of AD and TP. LP includes one cleavable site (CS).
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) DNA sequence (I) that encodes IP;
(2) expression vector (II) containing DNA (Ia) that encodes a hybrid polypeptide of IP and TP; and
(3) method for producing TP by transforming cells with (II), recovering TP from the cell and purifying it by exploiting the **ligand**-binding affinity of IP.
USE - IP is used for detection, production and purification of recombinant **proteins**, i.e. TP-IP fusion **proteins**.
ADVANTAGE - IP makes it possible to purify any **protein**, by

antibody-based **affinity chromatography**, even where the **protein** is not antigenic and can not normally be purified by **affinity** methods. The presence of many epitopes in IP increases the detection limit of fusion **proteins** containing IP.
Dwg.0/3

TECH UPTX: 20010528
TECHNOLOGY FOCUS - BIOLOGY - Preferred Peptide: Each AD contains no more than 10 amino acids (aa) and has at least 50% hydrophilic residues and has at least one each of hydrophilic and aromatic residues or contains Arg, Pro, Glu, Asp, Phe and/or Ile. CS is cleaved by a sequence-specific proteolytic agent at an aa residue next to TP, particularly by enterokinase (best), factor Xa or thrombin. IP may include a spacer domain (SD) between two or more AD, or between the AD component and LS and/or a metal-chelating sequence attached to the AD component, particularly a sequence containing His and/or Gly, or a combination of many or **alternating** His. One preferred IP has the formula
X20-(X1-Y-K-X2-X3-D-X4)n-X5-(X1-Y-K-X7-X8-D-X9-K)-X21
X20 and X21 = hydrogen or bond;
each X1 and X4 = bond or at least one aromatic or hydrophilic aa;
each X2, X3, X7 and X8 = aromatic or hydrophilic aa;
X5 = bond or spacer containing at least one of aa, His, Gly or the combinations His-Gly-His or -(His-X)m-;
m = 1-6;
X = Gly, His, Tyr, Trp, Val, Leu, Ser, Lys, Phe, Met, Ala, Glu, Ile, Thr, Asp, **Asn**, Gln, Arg, Cys and Pro;
X9 = bond or Asp;
n = 2.
Preferred **Proteins**: TP can be any **protein**, e.g. an enzyme, storage **protein**, actin, antibody, growth factor and hormone.
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: A gene for TP was ligated to synthetic sequences encoding IP and the product cloned into a suitable vector for expression in usual host cells (prokaryotic or eukaryotic). The transformed cells were grown and the recombinant fusion **protein** recovered by **affinity chromatography** on antibodies specific for the AD component or by metal-ion **affinity chromatography** (if IP includes His and/or Gly residues). The recovered fusion **protein** is then treated with a specific proteolytic agent and the TP released **separated** by gel filtration or dialysis (it is generally much larger than the IP fragment and other contaminants). The only contaminant in TP will be any uncleaved fusion **protein** and this is removed by **affinity chromatography** on the same column as used for initial **separation** of fusion **protein**.

L13 ANSWER 13 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2001-218352 [22] WPIDS
DNC C2001-065186
TI Screening enhanced, reduced intein cleavage activity by expressing randomly mutagenized intein DNA and screening cleavage activity using varying growth medium and conditions, or by assay with chemical, respectively.
DC B04 **D16**
IN BELFORT, G; BELFORT, M; DERBYSHIRE, V; WOOD, D; WU, W
PA (HEAL-N) HEALTH RES INST
CYC 88
PI WO 2001012820 A1 20010222 (200122)* EN 103p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ

NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 2000067817 A 20010313 (200134)
ADT WO 2001012820 A1 WO 2000-US22581 20000817; AU 2000067817 A AU 2000-67817 20000817
FDT AU 2000067817 A Based on WO 200112820
PRAI US 1999-149257P 19990817
AB WO 200112820 A UPAB: 20010421
NOVELTY - Screening (M1) for enhanced or reduced intein cleavage activity involves subjecting intein DNA to random mutagenesis, expressing the intein DNA with a reporter and screening for elevated or reduced intein cleavage activity using varying growth medium and conditions, or by an assay with a chemical that plays a part in a cell metabolic and/or biochemical cycle, respectively.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a non-naturally occurring intein (I) or cleavage or cleavage and splicing group having splicing activity and/or controllable cleavage activity;

(2) a **protein** (II) including (I);

(3) an isolated nucleic acid molecule (III) encoding (I) or (II);

(4) a vector (IV) containing (III);

(5) a host cell (V) transformed with (IV);

(6) producing a **protein** (M2) involves preparing (II) and subjecting it to cleavage conditions, preferably a fusion of (I) and (II) is prepared after which the fusion is subjected to cleavage conditions;

(7) preparation of (I);

(8) determining (M3) amino acid residues in an intein that play a role in cleavage activity involves:

(a) deleting and/or changing amino acids in the intein to obtain an **altered** intein;

(b) preparing fusion of the **altered** intein and a reporter;

(c) selecting for reduced intein cleavage activity using an assay with a chemical that plays a part in:

(i) a cell metabolic; and/or

(ii) biochemical cycle; and/or

(d) selecting for elevated intein cleavage activity using selective varying growth medium and conditions;

(9) recombinant molecule (VI) encoding a fusion **protein** containing (III), where the intein is inserted in a specific region in the **protein** such that activity of the intein is retained in a control-specific manner; and

(10) a recombinant molecule (VII) encoding I-TevI fused with an intein such that, upon expression of the fusion construct, I-TevI is expressed in amounts suitable for **protein** purification.

USE - (I) is useful for purifying a desired **protein** which involves preparing a fusion polypeptide comprising a binding **protein** portion, (I) and a desired **protein** portion, binding the fusion to a binding group, subjecting the intein to cleavage conditions and **separating** the desired **protein**. Binding of the fusion to the binding group involves binding the fusion to an **affinity** matrix, and the **separation** step involves subjecting the **affinity** matrix to a pH and/or temperature shift and eluting the desired **protein** (claimed). The **protein** purification activity of (I) was demonstrated by the purification of

I-TevI, a lethally toxic T4 td intron-encoded homing endonuclease. The fusion gene I-TevI::SM::CBD with the intein N-terminal to Cys164 was cloned into pET28a. A non-spliceable control, I-TevI::SMAA, in which the His-**Asn** dipeptide at the C-terminus of the SM mini-intein was mutated to Ala-Ala, was also cloned into pET28a to test the toxicity of the unspliced precursor. When the plasmids were transformed into BL21(DE3), there were no transformants for pET28-I-TevI::SM::CBD but many transformants for pET28-I-TevI::SMAA. Restored toxicity suggested leaky expression of I-TevI. To reduce the leaky expression of I-TevI::SM::CBD, the stain BL21(DE3)pLysS was used, which has more stringent control over T7 polymerase by inhibiting its activity with T7 lysozyme expressed from the pLysS plasmid. When the pET28-I-TevI::SM::CBD plasmid was transformed into BL21(DE3)pLysS, many transformants with the correct wild-type sequence were obtained. The results indicate that I-TevI toxicity had been suppressed to a tolerable level by intein inactivation.

ADVANTAGE - The method provides highly active, controllable self cleaving inteins. The system provides a way to accelerate C-terminal cleavage reaction without N-terminal cleavage. Small mutant inteins with desirable splicing or cleaving properties suitable for **affinity separations** can be obtained. Through the development of a genetic screen, mutant mini-inteins were isolated with restored splicing activity and enhanced, controllable cleavage activity. Mutant mini-inteins isolated using this screen have elevated activities in vivo and in vitro, and form the basis of a pH- and temperature-dependent **protein** purification system. By selecting mini-inteins that display rapid, isolated C-terminal cleavage, the novel system generated a pH-sensitive mutant intein, which obviates the need for reducing reagents and additional purification steps, and has advantageous size and stability characteristics. The specific behavior of the inteins is further advantageous in exhibiting a 20- to 40-fold increase in activity between pH 8.5 and 6.0. These pH values are relatively mild, decreasing the potential for damage to the product **protein** due to pH induced denaturation, and thus allowing the recovery of pure **protein** with minimal damage.

Dwg.0/26

TECH UPTX: 20010421

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: Preparation of (I) involves subjecting intein DNA to random mutagenesis, expressing the intein DNA with a reporter and screening for elevated intein cleavage activity using varying growth medium and conditions.

Preferred Intein: (I) Is a truncated intein whose cleavage activity is controllable by varying a physical condition such as pH or temperature or varying a chemical condition such as ion concentration, presence or absence or varying both the physical and chemical conditions. Preferably (I) cleavage activity is controllable by at least two of the following conditions: varying pH; varying temperature; varying ion concentration; presence or absence. More preferably, (I) cleavage activity is controllable by varying pH and temperature. (I) As described above is also a mutant intein obtained from random mutagenesis of truncated intein, followed by selection based on a growth phenotype. (I) has C-terminal cleavage and has C-terminal hisitidine and is preferably a truncated Mtu intein. The cleavage rate of (I) is determined by an enzymatic reaction and not by a chemical reaction. Preferably (I) has the endonuclease domain deleted.

Preferred **Protein**: (II) Comprises a polypeptide of interest and the intein which is in an inter domain of the polypeptide of interest. (II) Comprises a binding **protein** portion, the intein and a reporter **protein** portion, in which the intein separates the

binding **protein** portion and the reporter **protein** portion. The reporter **protein** is an enzymatic assay **protein**, a **protein** conferring antibiotic **resistance**, or a **protein** providing a direct colorimetric assay. The reporter **protein** is a thymidylate synthase, beta-galactosidase, galactokinase, **alkaline** phosphatase, luciferase, beta-lactamase or green fluorescent **protein**. **Alternately**, (II) comprises a binding **protein** portion, the intein, and a **protein** of interest portion, where the intein separates the binding **protein** portion and the **protein** of interest portion. (II) comprises a desired polypeptide and the intein:
(i) as an external or internal fusion, where the intein is located before a serine, threonine or cysteine residue of the desired polypeptide;
(ii) where the intein and desired polypeptide and separated by a serine, threonine or cytosine residue;
(iii) where the C-terminal histidine or **asparagine** or histidine-**asparagine** of the intein is immediately followed by the initial methionine of the desired polypeptide; or
(iv) where the initial methionine of the desired polypeptide has been eliminated.
(II) Which comprises a desired polypeptide and an intein containing a C-terminal histidine, has the C-terminal histidine or **asparagine** or histidine-**asparagine** of the intein, immediately followed by the second amino acid (a lysine) of the desired polypeptide.
Preferred Method: In (M1), the cleavage rate is determined by an enzymatic reaction and not by a chemical reaction. The random mutagenesis process involves amplifying intein DNA which codes for a truncated intein, using a polymerase. Screening reduced intein cleavage activity involves using a trimethoprim gradient assay with the chemical trimethoprim that plays part in the folic acid cycle. In (M2), the cleavage conditions allow about 90% cleavage in four hours at 37degreesC about 12 hours at 25degreesC or about 150 hours at 4degreesC. Preferably, the cleavage conditions allow about 90% cleavage in about 6-8 hours at 23degreesC. Also, the cleavage conditions allow cleavage at a physiological pH of 6-8.5. Preferably, the **protein** or fusion is prepared recombinantly. By preparing a vector containing DNA encoding the **protein** or the fusion, transforming host cell with the vector and expressing the DNA in the host cell.
Preparation of (I) involves performing random mutagenesis process by amplifying intein DNA using a polymerase. In (M3), the fusion is prepared by expressing the **altered** intein with the reporter, and deleting and/or changing amino acid in the intein is by random mutagenesis. Preferably, the amino acid(s) that is deleted and/or changed immediately precedes a conserved amino acid such as serine, cysteine or threonine. The reporter used in the method is thymidylate synthase.
Preferred Host Cell: (V) is Escherichia coli.
Preferred Vector: (IV) is a plasmid.
Preferred Recombinant Molecule: In (VI), the intein is inserted in one or more of a N-terminal domain, a C-terminal domain, a joining segment, an interface between the N-terminal domain and the joining segment or an interface between the joining segment and the C-terminal domain. Preferably, the intein is inserted, N-terminal to a zinc finger region or Cys rich region and in the interface between the joining segment and the C-terminal. (VII) comprises pET28-I-TevI::SM::CBD plasmid.

L13 ANSWER 14 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-339682 [29] WPIDS
DNC C2000-103135
TI An improved method of **affinity separation**, useful for

testing the presence of a target molecule and yielding pure samples of the target molecule, includes an immobilized **affinity ligand** with one or more **modified asparagine** residues.

DC B04 **D16**

IN HOBER, S; UHL, N M; HOBER, S E; UHLEN, M

PA (AFFI-N) AFFIBODY TECHNOLOGY SWEDEN AB; (GARD-I) GARDNER R; (AFFI-N) AFFIBODY AB

CYC 91

PI WO 2000023580 A1 20000427 (200029)* EN 49p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9963532 A 20000508 (200037)
NO 2001001969 A 20010619 (200143)
EP 1123389 A1 20010816 (200147) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MK RO SI
JP 2002527107 W 20020827 (200271) 50p
ZA 2001003252 A 20020925 (200275) 63p

ADT WO 2000023580 A1 WO 1999-GB3484 19991021; AU 9963532 A AU 1999-63532 19991021; NO 2001001969 A WO 1999-GB3484 19991021, NO 2001-1969 20010420; EP 1123389 A1 EP 1999-950942 19991021, WO 1999-GB3484 19991021; JP 2002527107 W WO 1999-GB3484 19991021, JP 2000-577291 19991021; ZA 2001003252 A ZA 2001-3252 20010420

FDT AU 9963532 A Based on WO 200023580; EP 1123389 A1 Based on WO 200023580; JP 2002527107 W Based on WO 200023580

PRAI GB 1998-23071 19981021

AB WO 200023580 A UPAB: 20000617
NOVELTY - A method of **affinity separation** where the **affinity ligand** is an immobilized proteinaceous **ligand**, with one or more **modified asparagine** (**Asn**) residues, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of stabilizing an affinity **ligand** by **modifying** one or more of its **Asn** residues;

(2) a method of preparing a combinatorial library of **proteins** which have been rendered less **sensitive** to **alkaline** pH by **modification** of one or more of its **Asn** residues before it is randomized;

(3) a method of phage display where a **protein** expressed on the phage surface has one or more of its **Asn** residues **modified** in a separate step to the **modification** of the **proteins** binding characteristics;

(4) a method of making a stabilized combinatorial **protein**, comprising **modifying Asn** residues in a **protein** to increase stability of the **protein** in **alkaline** conditions, and randomizing the **protein** to **modify** its binding characteristics;

(5) a combinatorial **protein** where the stability of the **protein** in **alkaline** conditions has been increased by **modifying** one or more of its **Asn** residues;

(6) a fusion **protein** comprising a region with one or more **Asn** residues which have been **modified**, and a region comprising a randomized **protein** molecule having a specific binding domain;

(7) an Albumin Binding **Protein** (ABD), or its fragments or derivatives, where one or more native **Asn** residue have been replaced by a less **alkaline sensitive** amino acid;

(8) a nucleic acid molecule encoding the affinity **ligand**, or combinatorial or fusion **protein**; and

(9) a host cell expressing the affinity **ligand**, or combinatorial or fusion **protein**.

USE - The method is useful for testing the presence of a target molecule. It also provides excellent purification methods, yielding samples of a target molecule having good purity. The method is further useful for generating **ligands** with novel, **modified** or enhanced binding characteristics. The fusion **proteins** are useful as an immobilized **ligand** in **affinity chromatography** (claimed).

ADVANTAGE - **Affinity chromatography** involving an immobilized **protein ligand** has advantages over ion-exchange or gel-filtration **chromatography**. The very selective nature of interactions between or involving **affinity** binding molecules, particularly **proteins**, makes them ideal for purification/**separation** techniques. This method can provide samples of high purity as quickly and cheaply as possible, and is less susceptible to standard cleaning methods, particularly **alkaline** pH.

Dwg.0/7

TECH UPTX: 20000617

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred **Protein**: The **protein** or **ligand** may have all of its **Asn** residues **modified**, preferably by replacing it with a less **alkaline-sensitive** amino acid, such as lysine, aspartic acid or leucine. **Modification** may also include other chemical derivatization, e.g. using a protecting group. The **Asn** residues on the surface of the three-dimensional structure of the **ligand** or **protein** may also be **modified**. The affinity **ligand** or **protein** is the Albumin-Binding **Protein**, or its fragment or derivative, or a combinatorial **protein**, preferably a randomized **protein** selected by expression in a surface display library. The combinatorial **protein** may be derived from an immunoglobulin molecule, or its fragment or derivative, Staphylococcal **protein** A (SPA), or its fragment, domain or derivative, or a DNA binding **protein**, or its fragment or domain. The combinatorial **protein** may be domain Z or its derivative. One region of the fusion **protein** is ABD and the second region is domain Z or its derivative.

L13 ANSWER 15 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 1992-349209 [42] WPIDS
DNN N1992-266392 DNC C1992-155067
TI Monoclonal antibodies against LPS core epitope(s) of Gram-negative bacteria - for diagnosing, preventing and treating Gram-negative bacterial infections.
DC B04 D13 D15 **D16** J04 S03
IN BARCLAY, G R; DI, PADOVA F; GRAM, H; POXTON, I R
PA (SANO) SANDOZ LTD; (SANO) SANDOZ PATENT GMBH; (COMM-N) COMMON SERVICES AGENCY; (ESSO) EXXON CHEM PATENTS INC
CYC 37
PI WO 9216624 A1 19921001 (199242)* EN 82p
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL OA SE
W: AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US

AU 9212611 A 19921021 (199303)
EP 576439 A1 19940105 (199402) EN
R: AT CH DE DK ES FR GB IT LI NL SE
JP 06505867 W 19940707 (199431) 28p
US 5858728 A 19990112 (199910)
EP 576439 B1 19990506 (199922) EN
R: AT CH DE DK ES FR GB IT LI NL SE
DE 69229110 E 19990610 (199929)
ES 2131526 T3 19990801 (199937)
JP 3328277 B2 20020924 (200264) 47p

ADT WO 9216624 A1 WO 1992-EP380 19920222; AU 9212611 A AU 1992-12611 19920222, WO 1992-EP380 19920222; EP 576439 A1 EP 1992-904901 19920222, WO 1992-EP380 19920222; JP 06505867 W JP 1992-504918 19920222, WO 1992-EP380 19920222; US 5858728 A Cont of US 1993-119046 19930930, US 1996-647144 19960509; EP 576439 B1 EP 1992-904901 19920222, WO 1992-EP380 19920222; DE 69229110 E DE 1992-629110 19920222, EP 1992-904901 19920222, WO 1992-EP380 19920222; ES 2131526 T3 EP 1992-904901 19920222; JP 3328277 B2 JP 1992-504918 19920222, WO 1992-EP380 19920222

FDT AU 9212611 A Based on WO 9216624; EP 576439 A1 Based on WO 9216624; JP 06505867 W Based on WO 9216624; EP 576439 B1 Based on WO 9216624; DE 69229110 E Based on EP 576439, Based on WO 9216624; ES 2131526 T3 Based on EP 576439; JP 3328277 B2 Previous Publ. JP 06505867, Based on WO 9216624

PRAI GB 1991-5292 19910313

AB WO 9216624 A UPAB: 19931115

The following are claimed (A) monoclonal antibodies which recognise an epitope in the core region of the bacterial lipopolysaccharide (LPS) mol. and which are cross-protective against endotoxaemia caused by at least two different Gram -negative bacterial strains having different core structures, (B) hybridoma cell lines producing antibodies of type (A), (C) LPS- binding **proteins** having at least one antigen-binding site comprising at least one domain which comprises in sequence, the hypervariable hCDR1, hCDR2 and hCDR3 regions, where hCDR1 has the sequence Asp-Tyr-Tyr-Met-Thr, hCDR2 has the sequence Leu-Ile-Arg-**Asn**-W-Arg-**Asn**-Gly-Asp-Thr-Ala-Glu-Tyr-Ser-Ala- Ser-Val-X (W = Lys or Tyr, X = Lys or Arg) and hCDR3 has the sequence Gln-Gly-Arg-Gly-Tyr-Thr-Leu-Asp-Tyr, (D) DNA constructs coding for hCDR1, hCDR2 and hCDR3 in sequence, (E) DNA constructs encoding a heavy chain or fragment and comprising (a) a first part encoding a variable domain comprising **alternate** framework and hypervariable (hCDR1-hCDR2-hCDR3) regions, and (b) a second part encoding a heavy chain constant part or fragment, followed by a non-sense codon, (F) DNA constructs coding for the hypervariable regions lCDR1, lCDR2 and lCDR3 in sequence, where lCDR1 has the sequence Arg-Ala-Y-Z-**Asn**-Ile-**Asn**-Ile-Trp-Leu-Ser (Y = Ser or Arg, Z = Gln or Leu), lCDR2 has the sequence Lys-Ala-Ser-**Asn**-Leu-His-Thr and lCDR3 has the sequence Leu-Gln-Gly-Gln-Ser-Tyr-Pro-Arg-Thr (G) DNA constructs encoding a light chain or fragment and comprising (a) a first part encoding a variable domain comprising **alternate** framework and hypervariable (lCDR1-lCDR2-lCDR3) regions, and (b) a second part encoding a light chain constant part or fragment, followed by a non-sense codon.

USE - The antibodies and LPS-binding **proteins** are useful for prevention or treatment of Gram-negative endotoxaemia. The **proteins** are also useful for removing LPS from biological fluids by **affinity chromatography**. The labelled **proteins** are also useful for diagnostic purposes, e.g. for localising sites of infection or detecting bacterial contamination of water, foods etc. The DNA constructs may be used to produce humanised forms of the antibodies

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L13 ANSWER 16 OF 16 WPIDS (C) 2003 THOMSON DERWENT
AN 1992-064885 [08] WPIDS
DNC C1992-029742
TI Constructs for purificn. directed cloning of peptide(s) - comprise binding **protein**, esp. carbonic anhydrase, and pref. polypeptide linked by interconnecting peptide comprising cleavage site.
DC B04 **D16**
IN COOLIDGE, T R; SCHUSTER, S M; STOUT, J; VAN, HEEKE G; WAGNER, F; WYLIE, D E; VANHEEKE, G; COOLRIDGE, T R
PA (BION-N) BIONEBRASKA INC
CYC 22
PI WO 9201707 A 19920206 (199208)*
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
W: AU CA FI JP NO
AU 9189155 A 19920218 (199222)
NZ 238812 A 19930127 (199310)
ZA 9105307 A 19930224 (199315) 82p
EP 539530 A1 19930505 (199318) EN 84p
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
JP 06505624 W 19940630 (199430) 20p
EP 539530 A4 19930915 (199527)
AU 662302 B 19950831 (199543)
US 5595887 A 19970121 (199710) 27p
EP 539530 B1 20000202 (200011) EN
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
DE 69131961 E 20000309 (200019)
US 6127150 A 20001003 (200050)
JP 3129437 B2 20010129 (200113) 32p
CA 2087261 C 20021224 (200306) EN
ADT AU 9189155 A AU 1991-89155 19910624, WO 1991-US4511 19910624; NZ 238812 A NZ 1991-238812 19910701; ZA 9105307 A ZA 1991-5307 19910709; EP 539530 A1 WO 1991-US4511 19910624, EP 1992-902529 19910624; JP 06505624 W WO 1991-US4511 19910624, JP 1992-500610 19910624; EP 539530 A4 EP 1992-902529 ; AU 662302 B AU 1991-89155 19910624; US 5595887 A US 1990-552810 19900716; EP 539530 B1 WO 1991-US4511 19910624, EP 1992-902529 19910624; DE 69131961 E DE 1991-631961 19910624, WO 1991-US4511 19910624, EP 1992-902529 19910624; US 6127150 A Div ex US 1990-552810 19900716, Cont of US 1996-680004 19960715, US 1997-927128 19970905; JP 3129437 B2 WO 1991-US4511 19910624, JP 1992-500610 19910624; CA 2087261 C CA 1991-2087261 19910624, WO 1991-US4511 19910624
FDT AU 9189155 A Based on WO 9201707; EP 539530 A1 Based on WO 9201707; JP 06505624 W Based on WO 9201707; AU 662302 B Previous Publ. AU 9189155, Based on WO 9201707; EP 539530 B1 Based on WO 9201707; DE 69131961 E Based on EP 539530, Based on WO 9201707; US 6127150 A Div ex US 5595887; JP 3129437 B2 Previous Publ. JP 06505624, Based on WO 9201707; CA 2087261 C Based on WO 9201707
PRAI US 1990-552810 19900716; US 1996-680004 19960715; US 1997-927128 19970905
AB WO 9201707 A UPAB: 19931006
Construct (A) comprises: a binding **protein** (BP) or a modified functional version of a BP, and a variable fused polypeptide (VFP). The BP and VFP are tandemly coupled together by an interconnecting peptide, and the BP is an enzyme-like **protein**. More specifically, the VFP is a single peptide unit or a gp. of peptide units tandemly linked together by an intraconnecting peptide, and the peptide unit is a single prod. peptide, or a gp. of prod. peptides tandemly linked together by an

inner-connecting peptide. The aminoacid (AA) sequences of the inter, intra and innerconnecting peptides are not available for cleavage in the BP or prod. peptides. The BP has highly specific binding to a low mol.wt. **ligand**, has **ligand** binding site(s) and a **ligand** soln. dissociation constant of no greater than 10 power-7 M.

USE/ADVANTAGE - Recombinant DNA technology with a **ligand** immobilised **affinity sepn.** technique allows large scale, low cost and highly efficient biological synthesis. The construct includes a large binding **protein** so subversion of the identify of the prod. peptide by the expression mechanism of the host cell is not permitted. Variation in expression efficiency is minimised. The high binding constant of the binding **protein** for the **ligand** enables clean, complete **sepn.** of the VFP from other constituents. The low mol.wt. of the immobilised **ligand** achieves a high capacity and large scale of **sepn.** per unit wt. of immobilised support. When the BP and **ligand** are an enzyme and inhibitor there is no spurious enzymatic clearage to produce undesirable side prods.. Repetitions gene sequences can be unstable in the host cell, hence the use of the multiple unit concept and codon diversity in the gene segment for the VFP.

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